ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.

AT

25 CENTS PER COPY
SUBSCRIPTION PER YEAR, 12 NUMBERS, $2.50.

JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

VOL. III WASHINGTON, D. C., JANUARY 15, 1915 NO. 4

OBSERVATIONS ON THE LIFE HISTORY OF AGRILUS BILINEATUS

By ROYAL N. CHAPMAN,

Graduate Assistant, Division of Entomology, Department of Agriculture, University of Minnesota

INTRODUCTION

At the present time the two-lined chestnut borer, *Agrilus bilineatus* Weber, is commonly associated with the death of many oaks (*Quercus* spp.) in the southeastern part of Minnesota. In 1885 reports called attention to the damage done by this insect on oaks in Massachusetts, and since that time they have been frequently mentioned as enemies of the chestnut (*Castanea dentata*) and oak. It was not until 1897 that F. H. Chittenden [1] described the adult, the larva, and the pupa, in connection with a brief summary of its life history, so far as it was known at that time. In this article it was stated that the adults appeared in the District of Columbia from May to the middle of June and laid their eggs on trees and that the larvæ worked under the bark across the grain of the wood, making a burrow from 6 to 10 inches in length, and by the next spring had constructed a chamber in the bark of living trees, where the pupal stage of about two weeks was passed. Although the name of this beetle has been common in current entomological literature, nothing of note has been added to the knowledge of its life history since Chittenden's article.

During recent years in the neighborhood of St. Paul and Minneapolis great numbers of oaks, many of them on valuable residence property, have been killed, and their death has commonly been attributed to this pest. In some of the outlying country districts areas of several acres in extent have been completely devastated, leaving the land treeless.

The present work was begun at the University of Minnesota during the fall of 1913 at the suggestion of Prof. O. W. Oestlund, of the Department of Animal Biology, under whose direction the problem was outlined and the work on the larval and pupal stages begun. In the spring of 1914 the work was continued at the Minnesota Agricultural Experi-

[1] Chittenden, F. H. Insect injury to chestnut and pine trees in Virginia and neighboring States. *In* U. S. Dept. Agr., Div. Entom. Bul. 7, n. s., p. 67–71. 1897.

ment Station under the direction of Prof. A. G. Ruggles, in charge of the Section of Spraying and Tree Insects, Division of Entomology.

The aim of this paper is to report new observations on the life history and ecologic relations of *Agrilus bilineatus*. The descriptions of the adults, larvæ, and pupæ, already referred to, are so well known to entomologists that they will not be repeated. The drawings reproduced in Plate XXXVIII of the beetles, pupæ, larvæ, and eggs were made at the Department of Animal Biology under the direction of the author. The photographs reproduced in Plate XXXIX were made at University Farm by the Experiment Station photographer.

The egg-laying habits have been described in this article in some detail because they have not been known to literature, and the same is true of the leaf-eating habits of the adults. The same may be said of the eggs and newly hatched larvæ. Some of the observations of the life history of the larvæ have not agreed with previous descriptions, and these, together with a few additional notes on habits, are included, while others, together with the details of the pupal stage, will be deferred to a later report.

ECOLOGY

The four common species of oak in the southeastern section of Minnesota, *Quercus alba* L., *Q. macrocarpa* Michx., *Q. rubra* L., and *Q. coccinea* Wang, are subject to infestation with *Agrilus bilineatus*. It seems that the members of the black-oak group are slightly more susceptible to attack than those of the white-oak group, but in localities where the infestation is severe none of the species is exempt.

In some cases the adult borers appear to prefer trees of a certain locality, or, in other cases, certain individual trees, to others even in the immediate vicinity. In general, this preference is associated with a weakened condition of the trees, but this is by no means universal. The environmental conditions of certain localities, such as drought, crowded pasturage, or cultivation, have made nearly all the trees subject to infestation by the two-lined chestnut borer, possibly because of a general weakened condition. In other cases individual trees weakened by injury or disease have been attacked, while in still other cases trees which show no signs of weakened vitality are attacked and killed by this insect.

It has often been found that the shoestring fungus, *Armillaria mellea* Vahl, has apparently been the cause of the weakened condition of the trees, and the chestnut or oak borers have followed it. In fact, it has sometimes appeared that *A. mellea* was the primary cause of the death of the trees and that the Agrilus beetle was of only secondary importance. An example of this was found in the neighborhood of Lake Elmo, Minn., where a few dead trees were found with the fungus Armillaria, but with no traces of larvæ of the two-lined chestnut borer. These observations, together with others which showed the shoestring fungus on practically every tree on which the Agrilus beetles were ovipositing, made

it seem probable that the fungus was the primary factor in causing the death of the trees.

In the vicinity of Robbinsdale, Minn., and in other localities, the Armillaria fungus is present, but is not so apparent, and all the dead trees show traces of oak borers. Furthermore, many trees with dead trunks started up from the roots the following year and gave every evidence of suffering from the girdling of the cambium layer rather than from a root infestation with the fungus.

At Robbinsdale a tree was observed on July 27 with its leaves withering as if it had been scorched, a characteristic of trees infested with *Agrilus bilineatus*. Three days before (July 24) this tree had appeared perfectly normal, but on the 27th the entire trunk of the tree was being girdled by the beetles. It was grubbed and its roots were examined by Mr. F. J. Piemeisel, of the Division of Plant Pathology, who stated that the fungus *Armillaria mellea* was not present and could have nothing to do with the death of the tree and that the root system seemed perfectly healthy. Other trees examined since gave similar evidence.

It has not yet been possible to show any relation between the amount of fungus present on a tree and the severity of the attack by the beetles. On land that was being cleared for cultivation near Lake Elmo 40 apparently healthy trees were attacked by *Armillaria mellea*, and none were found to be free from it. Furthermore, W. H. Long states [1] that a large percentage of the oaks examined by him in the eastern section of the United States have been found to be infested with the fungus. If this is true also in Minnesota and the presence of the fungus is taken as evidence of the low vitality of the tree, all but a small percentage of the oaks in Minnesota are now susceptible to attack by *Agrilus bilineatus*, and in all these cases the beetles must be looked upon as of only secondary importance. This, however, would possibly place undue weight on the mere presence of *Armillaria mellea*.

As mentioned above, in a few cases in the vicinity of Lake Elmo the death of the trees may be due to *Armillaria mellea* alone. In the majority of cases the fungus was present, but the trees were girdled by the two-lined chestnut borer, seemingly a hastening factor, at least, in the death of the trees. There still remains the possibility that the fungus was the primary factor. There are cases, as already mentioned, where the borers alone have attacked and killed apparently normal trees when the fungus was not present. The economic importance of this condition can hardly be overemphasized, for it means that the Agrilus beetles, in spite of their supposed preference for unhealthy trees, chose one healthy tree when many trees infested with the fungus were available, indicating that the interrelation between the *Armillaria mellea* and the *Agrilus bilineatus* may not be of such primary importance as would appear at first.

[1] Long, W. H. The death of chestnuts and oaks due to Armillaria mellea. U. S. Dept. Agr. Bul. 89, p. 4. 1914.

THE ADULTS

On the 17th of June the first observations of the adult *Agrilus bilineatus* were made in the vicinity of Lake Elmo, Minn. Early in the afternoon two or three could sometimes be seen at one time on a dying tree. On the same day an adult was taken from its pupal cell in the bark, and a larva was found preparing to pupate. Since no adults were seen two days earlier while the author was collecting in the same locality, it seems that these observations fix the appearance of the adults quite definitely for this section in a normal year.

The adult borers increased in numbers until they reached their greatest abundance about the 1st of July, when as many as 10 were seen at one time on a small area of bark. In the afternoon of June 26, 80 specimens were collected near Savage, Minn. There was a noticeable decline in numbers after the first week in July, and by July 20 the last record of the adults was made. Continued careful search in the field after that time was not rewarded.

During the last days of the adults' flight many instances of apparent feebleness became evident. On one occasion, while watching Agrilus beetles, a female was seen to fly slowly toward a tree apparently intending to alight on it as usual, but the insect fell to the ground as if unable to cling to the bark. The borer then made a second attempt, which was also unsuccessful, and it was picked up from the ground where it was lying apparently exhausted. The behavior of other chestnut borers in the field and in the insectary leads to the belief that the last chapter of their history is marked by the feebleness of old age.

Agrilus adults lived about 12 days in the insectary, where they were kept in a cage inclosing an oak tree. The conditions were so favorable in the insectary that many details of the various habits could be studied to great advantage, and continuous observations throughout the season were made possible in spite of weather conditions.

The habits of the Agrilus beetles were governed with such regularity that a seemingly definite program was discovered which served as a guide for later observations. At no time during the season were beetles found, even on the sunny side of the trunks, until nearly noon. On July 7 in a place near Savage, Minn., where the adult borers were very abundant, a careful search was begun shortly after 9 o'clock in the morning and but one specimen was found at 9.30 a. m., a few at about 10 o'clock, and not until 11 o'clock were they found in their usual numbers. From this time until shortly after noon they increased in numbers; then they gradually disappeared until few were to be seen late in the day. The latest field observation was made about 6 o'clock. In the insectary the beetles were inactive in the bottom of the cage until about 8 a. m., when they would begin to fly about and feed on the foliage of the tree. Many were also observed courting during the early forenoon. The absence of the beetles from the tree trunks, which they frequent later in

the day, and their feeding habits as observed in the insectary, indicate the probability that the early part of the day is ordinarily spent in this way.

It was definitely proved that the adults of *Agrilus bilineatus* feed on the foliage. They usually eat around the margins of leaves, but also tear off the epidermis and sometimes eat nearly the entire leaf, including the midrib. Plate XXXIX, figure 1, shows a leaf on which four beetles had fed for 24 hours. They even ate the edge of a paper bag which was on the floor of the insectary, where most of the observations were made.

The difficulty of observing the feeding in the field was increased by the protective habits of the beetles. The flight to and from leaves or logs and tree trunks was ordinarily quite direct, but in some cases they hovered before lighting. When disturbed, however, they flew rapidly in a zigzag manner, which is probably of protective importance, as under such conditions it is almost impossible to follow them even with the eye. If they are startled while at rest, they fold their appendages and drop to the ground, feigning death. They dodge from side to side very quickly and run rapidly over any kind of surface. On one occasion a beetle was watched walking about inside a glass tube. It experienced no difficulty unless it tried to walk upside down upon the slippery concave surface, when it began to lose its footing. This was evidently not a new occurrence, for it immediately put one of its front tarsi to its mouth and apparently moistened it; it then reached this appendage back to rub it against the posterior ones. When all the tarsi had been treated in this way, the beetle ran about until it began to slip again, when the process was repeated.

On sunny days during the entire season males and females were courting and mating. These performances were often noticed on logs or woodpiles and on the foliage of small plants at the base of trees, as well as on tree trunks. The courting was usually abbreviated and sometimes wanting. Males were seen to fly from the air directly to females on the tree. At other times a male was seen to side-step to within an inch of a female and then spread its wings as if to fly before advancing farther. In one case a male stood near a female until she finished ovipositing and then mated with her. But it was not always the males that made the advances, for it was not uncommon to see females courting males, in which case they often found themselves ignored. In mating, the sexes were together from 2 to 12 minutes, with an average of about 4 minutes.

The females were ovipositing from June 19 to July 13. No record of ovipositing was obtained before 11 a. m. and but one after 5.30 p. m. While it was not so common to see females actually ovipositing, they could be seen nearly all the time during the hours of activity with their ovipositors out searching for places to oviposit. They laid the eggs in the bottoms of cracks, but not every crack was suitable. The females went carefully along, using their ovipositors as tactile organs, exploring every crevice. The insect often appeared to be in a great hurry and

rushed from one crack to another until the proper place was found. During the first week of July one female took 21 minutes from the time she lighted on a tree to find a place to lay her eggs. Not a minute of this time was wasted, and many cracks were rejected before the favorable one was located.

The cracks chosen for oviposition were usually ones that were quite deep. In one case a female used a crack which had evidently been made by lightning, but in all other cases a crevice at the bottom of a deep crack between ridges of the bark was chosen (Pl. XXXVIII, fig. 1). Since the bark is usually rougher on the trunk and larger limbs, especially near the ground, more favorable places to oviposit are found on these parts of the tree. Observations were made 25 feet from the ground, but few beetles were seen and none were looking for places to oviposit. In examining trees which had been killed, it was found in one case that an egg had been laid in a crevice at the axis of a small branch 41 feet 6 inches from the ground. On one tree which was very badly infested practically every branch more than 1½ inches in diameter had burrows on it. Many attempts were made to find whether the sunny side of trees was preferred to the shady side, but, so far as could be determined, the beetles showed no preference. From this evidence it seems that the eggs may be laid on any part of the tree which affords suitable cracks and that since such cracks are most numerous on the trunk, this is the usual place for ovipositing.

The females settled down when a favorable crack was found and were apparently motionless during oviposition. The beetles stood in any convenient position during the process, and there was no relation between the number of eggs laid and the length of time apparently spent in depositing them. Oviposition lasted from 1 to 5 minutes, and from 1 to 10 eggs were laid. It is probable that the number of eggs in a cluster depends upon the favorableness of the crevices in which they are deposited, because the females usually hasten to find another place as soon as one cluster has been laid. Just how many eggs are laid in all by one individual is not known.

THE EGG

Plate XXXVIII, figure 2, shows a very typical cluster of four eggs, the average number, on a bit of bark taken from the bottom of a crack, just as they appeared within an hour after they were deposited. One of the eggs lies entirely exposed, showing the typical form of an undisturbed egg, while the others illustrate how nicely they mass together and fit the irregularities of the crevices. It will be noticed that the eggs are not plump but have an unfilled, wrinkled appearance, which makes it possible for them to fit into crevices of all shapes. A typical egg, such as the exposed one in the illustration, is somewhat oval and measures about 940μ in length, 480μ in width, and about 300μ in thickness. The newly laid eggs were covered with a glistening substance which stuck them

The eggs were hatched in the laboratory in from 10 to 13 days. It was found that the outer membrane became dry and shriveled and even cracked in from 3 to 6 days, while the inner membrane became brown and the embryo seemed to develop at one side of the egg, which became plumper than the other side (Pl. XXXVIII, fig. 3).

THE LARVÆ

When the larvæ hatched, they broke through the egg membrane on the side toward the bark and immediately began to burrow. As a result, they were not exposed to view, and the eggshells were found filled with the frass which had been burrowed out at the start. In a few cases the eggs had been entirely loosened from the bark for examination, and none of the larvæ from these eggs succeeded in getting a burrow started, except in one case where the egg was artificially fastened to the bark before the larva left it. Since the eggs are stuck to the bark in the depths of cracks, they offer a certain resistance to the larva's efforts, which makes it possible for its mandibles to get hold of the bark and start the burrow.

The newly hatched larvæ (Pl. XXXVIII, fig. 4) measure only from 1 to 1½ mm. in length, but one was found capable of reaching the cambium layer in 24 hours by burrowing for 2¼ mm. The fact that the eggs were laid in the depths of cracks made it possible for the larvæ to reach the soft cambium layer by penetrating but a few millimeters of hard bark tissue. Having reached the cambium layer they started off in any convenient direction. Observations show that burrows made during the first instar often go obliquely across the grain of the wood or with the grain, the larvæ being indifferent as to whether they go up or down the tree. If care is taken in removing the bark when green, the tiny burrows can be traced to a widening of the burrow which marks the end of the first instar. The burrows measured showed that the larvæ had burrowed for a distance of 60 to 135 mm. when the first molt took place.

In most cases the widenings in the burrow occurred when the larvæ had gone into the wood, less often into the bark, and were again returning to the cambium layer. It is evident that these points marked the limits of instars and that the molting took place in these excavations in the wood, which were often two or three times the length of the larvæ. Places were found, however, where the burrows showed that the molt had been made in the cambium layer. The longest larva found in a burrow of the first instar measured 4.6 mm. in length, and the average width of the burrows was 270μ.

The burrows made during the second instar measured about 900μ in width and took about the same course through the cambium layer, but they were about twice as long. At the beginning of the third instar quite a different course was usually found, especially in green bark on the trunks of trees, where the burrows were almost always transverse to the grain of the wood. The burrows of the fourth instar were about 2 mm. in width

bark was thick these burrows were quite generally transverse to the grain of the wood. This condition, as well as the oblique course of some of the smaller burrows, is well shown in Plate XXXIX, figure 3.

At the close of the fourth instar the larva burrows out into the bark, if it is thick enough, and constructs a cell in which it hibernates. Here pupation takes place in the spring. These cells are found in the ridges of the bark on the trunk and larger limbs of the tree and in the wood on small, thin-barked trees and limbs. In constructing the cell, the larva burrows out to within a few millimeters of the surface of the bark, withdraws itself 2 or 3 mm., then turns about to one side and excavates around the posterior portion of its body until an oblong cell has been constructed. The portion of the burrow leading to the cell, as well as the short portion between the cell and the bark, is plugged with the frass loosened in making the excavation. It is evident that at least the portion of the frass at the outer end of the cell was never ingested, for if this were the case it would have passed out at the anus, which has been at the other end of the cell all the time. When the cell is complete, it measures about 10 mm. in length and about 2 or 3 mm. in width and contains the larva bent upon itself, ready for hibernation (Pl. XXXVIII, fig. 6).

The work of the larval life is well illustrated in Plate XXXIX, figure 4, which is a reproduction of a photograph of a limb in which the entire burrow is traced, from the shells of the eggs in a crevice of the bark to the larva in its pupal cell. The burrow was carefully marked with india ink, so that the black lines represent the exact width of the burrow in every case, and at the places where the larva entered the wood to molt the holes have been marked about with white ink for the sake of contrast. The larva in this case burrowed into the wood for a short distance at first, then returned to the cambium layer, where it burrowed about until it reentered the wood to molt. From the point where the larva entered the bark to the place it emerged from the wood after the first molt the burrow measures 69 mm. in length and 270μ in width.

It will be noticed that in each succeeding instar the burrow is much wider and longer, so that in the second instar the length is 103 mm. and the width is 900μ; in the third instar it is 210 mm. long and 1.21 to 1.56 mm. in width; and in the fourth and last instar the length is 456 mm. and the width is 1.96 to 2.15 mm. The total length of this particular burrow is 835 mm., or nearly 3 feet. Since the burrows made during the early instars are so small that they are hard to find, it seems likely that Chittenden's statement [1] that the complete burrow is only 6 to 10 inches in length was based on observations made on burrows which represented the last instar. Even these could hardly have been complete, for burrows of this instar nearly 2 feet long have been found. His statement that they are for the most part transverse to the grain of the wood makes it seem even more evident that those of the last instar were described, for, as Plate

[1] Chittenden, F. H. Loc. cit.

XXXIX, figure 3, shows, the transverse direction is the usual one in the last instar, especially when the bark is thick, while during the earlier instars the burrows run in a more oblique direction.

No evidence can be offered as to the duration of instars. Larvæ which were in the first stage were found from July 21 to August 13, mature larvæ were found in their pupal cells as early as August 7, while the intermediate stages were found throughout this period.

It was found that the larvæ in the last instar burrow from 2 or 3 to 23 mm. in 24 hours. Larvæ of the second instar burrow as far as 6 mm. in the same length of time. Upon consideration of these records it is not surprising that trees infested with *Agrilus bilineatus* appear to die suddenly when larvæ are to be found as numerous as shown by the burrows in Plate XXXIX, figure 3, where each one may consume cambium tissue equal to nearly twice its own bulk every 24 hours. In the section shown there were nine larvæ in approximately 1 square foot of bark, each burrowing across the cambium layer. It was also found that when the larvæ are so numerous that they confront each other, one or the other is eaten through as if it were merely cambium tissue. This may become an important economic factor, for cases have been observed in bark which was crowded with grubs where a number of dead larvæ were found with burrows passing through their abdomens or even their heads.

The slowest burrowing was found to be in the dry wood, where the tissue was evidently the toughest and of the least nutritive value. Trees with growing tissue offer the best opportunity for making extended burrows with great nutritive value to the larvæ and to the detriment of the tree. On the other hand, as soon as the tree dies from being girdled, the tissue becomes dry and offers more resistance to the burrowing and is of little nutritive value to the larvæ, which may die. A tree which was grubbed up on July 30, at which time it had just died, was examined on August 13. The dried bark, especially on the side exposed to the sun, contained shriveled larvæ, over 50 per cent of which were dead. Similar conditions were found in other trees that had died early in the season, when the dryness seemed to affect the larvæ more than later when they are in the pupal cells. This may also explain the condition described by Chittenden, who stated that burrows were found in trees, but no larvæ were present.[1]

In summarizing the work of the larvæ of the two-lined chestnut borer it is also of economic interest to note the wide distribution of the burrows on the tree, from the small branches less than an inch in diameter and between 40 and 50 feet from the ground down even to the roots, where in one case a larva was found constructing a pupal cell 11 inches below the surface of the ground.

---

[1] Chittenden, F. H. Loc. cit.

THE PUPA

The pupal stage of the life history has been studied for the most part in the laboratory. During the winter the larvæ were collected in their pupal cells and placed in wash bottles, which were then covered with cheesecloth. The larvæ were found to contract and straighten out in such a way as to face the end of the cell which is next to the bark. When contracted ready for casting the larval skin, the larvæ measured from 6 to 10 mm., instead of 18 to 24 mm., and were greatly swollen, with constrictions marking the posterior limits of the head, prothorax, and the location of the appendages. Two or three days before the larval skin was cast the posterior segments were collapsed and empty. When the pupa was ready, it began a series of wavelike dorsoventral bendings, which caused the skin to break on the dorsal side of the head and prothorax a little to one side of the middle. As these movements continued the skin was slipped gradually backward, collapsing as it left the posterior end of the body, until it was entirely off, when the pupa came to rest (Pl. XXXVIII, fig. 7). The mouth parts passed to the ventral side and seemed to act as a lever against the side of the cell in pushing the skin backward.

The pupal stage lasted about 10 days, during the latter part of which pigmentation began with the eyes, then the mouth parts, head, and thorax. The pupal skin was shed in much the same way as the larval skin, and the adult folded the wings, remaining inactive until the elytra were entirely pigmented. At first the movements of the adults were slow and uncorrelated as contrasted with the great activity later on. The beetle burrowed through the bark as soon as it acquired its full activity and escaped through a characteristic opening (Pl. XXXIX, fig. 2). The openings are always found on the ridges of bark and resemble the shape of the hole made by the larva when it first entered the bark.

Larvæ which had not yet pupated were collected as late as June 17, when adults were found making their way out from the pupal cells. From this it seems that the insect in this state normally pupates during the latter part of May and emerges from the cell about the middle of June.

PARASITIC ENEMIES

Two parasites were incidentally noticed. One, which was reared from the larvæ, was identified by Mr. S. A. Rohwer as a species of the genus Atanycolus, while another, unfortunately mutilated, which was reared from an egg, was placed by Mr. J. C. Crawford in the family Trichogrammidae.

CONTROL OF THE OAK BORER

The method of control heretofore recommended has been the cutting and burning of infested trees before the emergence of the adults in the spring. This is an effective method and needs emphasizing, for people are tempted to leave all the trees which show any signs of life, with the

hope that they will recover the next spring. These are the most dangerous trees, for, as has has been pointed out, the trees with sap are more favorable to the insects than the dry ones in which the larvæ are liable to dry up or starve.

The need of other methods, however, seemed imperative. During the past season the trunks and large limbs of some trees were sprayed with an iron-sulphate and lime-sulphur mixture, while others were sprayed with a Bordeaux mixture. This was done as a preventive measure during the egg-laying season and it seemed successful, as no beetles were seen on the trees which had been sprayed, even though the trees had been covered with beetles the day previous to this treatment. In contrast to this beetles were seen in great numbers throughout the season on the unsprayed trees near by.

Other experiments which are uuder way can not be reported until at least another season has passed, and greater opportunity has been offered to try out proposed methods of prevention and control.

PLATE XXXVIII

Fig. 1.—*Agrilus bilineatus:* Eggs in position in the bark of an oak tree. ½ natural size.

Fig. 2.—*Agrilus bilineatus:* Cluster of newly laid eggs. ×6.

Fig. 3.—*Agrilus bilineatus:* Eggs shortly before hatching. ×6.

Fig. 4.—*Agrilus bilineatus:* Newly hatched larva. ×6.

Fig. 5.—*Agrilus bilineatus:* Mature larva. ×6.

Fig. 6.—*Agrilus bilineatus:* Larva in its cell. Section made perpendicular to the surface of the bark. *A*, Point at which adult will emerge; *B*, burrow stopped with frass. ×6.

Fig. 7.—*Agrilus bilineatus:* Pupa in cell. Section made parallel to the surface of the bark. ×6.

Fig. 8.—*Agrilus bilineatus:* Adult female. ×6.

Fig. 9.—*Agrilus bilineatus:* Adult male. ×6.

Drawings by Helen A. Sanborn.


1
2
3
4
A
B
6
7
8
9
5

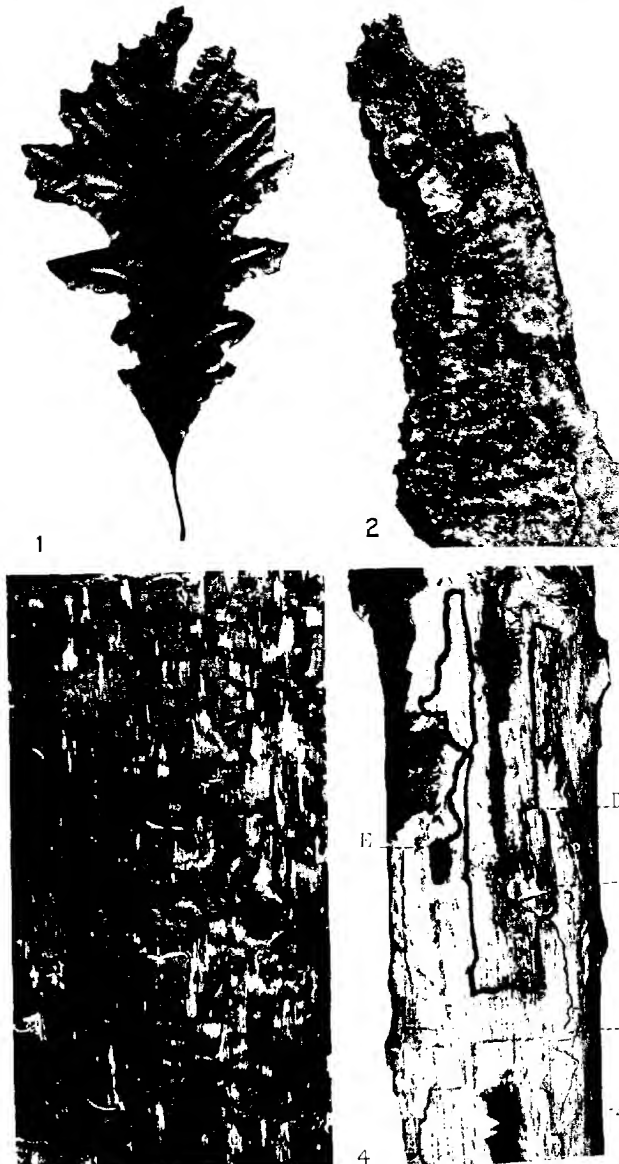

1
2
4
D
E
C
B
A

PLATE XXXIX

Fig. 1.—Leaf showing work of four Agrilus beetles in 24 hours.

Fig. 2.—Hole in bark made by adult Agrilus in emerging from pupal cell.

Fig. 3.—Larvæ of *Agrilus bilineatus* and their burrows.

Fig. 4.—Complete burrow of a larva of *Agrilus bilineatus*. *A*, Point at which larva hatched; *B*, beginning of second instar; *C*, beginning of third instar; *D*, beginning of fourth instar; *E*, pupal cell.

EFFECT OF DILUTION UPON THE INFECTIVITY OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO

By H. A. Allard,

Assistant Physiologist, Tobacco and Plant-Nutrition Investigations, Bureau of Plant Industry

In order to obtain some idea concerning the effect of dilution upon the infective power of the virus of the mosaic disease of tobacco in subsequent inoculations, the following experiments were made. A quantity of expressed sap from mosaic-diseased leaves was first passed through filter paper to remove the cell tissue, etc. Clean tap water was then used to bring the filtered virus to the required degree of dilution. All dilutions were accurately determined and inoculations immediately made from these. Young, vigorous plants growing in 3-inch pots in the greenhouse were used in all tests. In order to insure a thorough test of the infectivity of the diluted virus, a drop of the solution carried on the point of the needle was introduced with each puncture. Every leaf of any size on the plants, usually four or five, was inoculated in this manner at several points.

The plants were kept under observation for a long period after the first appearance of the disease in those groups treated with the original undiluted virus and the lower dilutions. This is virtually a quarantine period for the disease, since experience has shown that the incubation period of the mosaic disease is very uniform for simultaneous inoculations under any given set of conditions. A complete tabulation of all dilution experiments is given in Table I.

Table I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*

Date of inoculation.[a]	Number of plants.	Variety.	Degree of dilution.	Effect.
1913.				
May 6	10	Connecticut Broadleaf	Original undiluted virus	6 mosaic on June 1.
Do	10	do	1 part virus to 100 of water	7 mosaic on June 1.
Do	10	do	1 part virus to 1,000 of water.	Do.
Do	10	do	1 part virus to 10,000 of water.	4 mosaic on June 1.
Do	10	do	1 part virus to 1,000,000 of water.	All healthy on June 1.
Do	10	do	Tap water alone	Do.
May 9	20	do	Original undiluted virus	13 mosaic on May 26.
Do	20	do	1 part virus to 1,000 of water.	10 mosaic on May 26.
Do	20	do	1 part virus to 1,000,000 of water.	1 mosaic on May 20.
Do	10	do	Tap water alone	All healthy on May 26.

[a] All dilutions were prepared on the same day the inoculations were made.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. III, No. 4
Jan. 15, 1915
G—38

TABLE I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*—Continued

Date of inoculation.	Number of plants.	Variety.	Degree of dilution.	Effect.
1913.				
May 29	20	Connecticut Broadleaf	Original undiluted virus	11 mosaic on June 5.
Do	19	do	1 part virus to 1,000 of water.	17 mosaic on June 5.
Do	33	do	1 part virus to 1,000,000 of water.	All healthy on June 5.
Do	10	do	Tap water alone	Do.
June 12	15	do	Original undiluted virus	11 mosaic on June 18.
Do	21	do	1 part virus to 1,000 of water.	8 mosaic on June 18.
Do	21	do	1 part virus to 10,000 of water.	7 mosaic on June 18.
Do	30	do	1 part virus to 1,000,000 of water.	1 mosaic on June 18.
Do	10	do	Tap water only	All healthy on June 18.
June 21	30	20 Cuban 10 Connecticut Broadleaf	Original undiluted virus	26 mosaic on July 6 (18 Cuban, 8 Connecticut Broadleaf).
Do	30	15 Cuban 15 Connecticut Broadleaf	1 part virus to 1,000 of water.	20 mosaic on July 6 (11 Cuban, 9 Connecticut Broadleaf).
Do	33	18 Cuban 15 Connecticut Broadleaf	1 part virus to 10,000 of water.	12 mosaic on July 6 (4 Cuban, 8 Connecticut Broadleaf).
Do	20	10 Cuban 10 Connecticut Broadleaf	Tap water only	All healthy on July 6.
June 24	12	Cuban	Original undiluted virus	12 mosaic on July 6.
Do	20	do	1 part virus to 1,000 of water.	19 mosaic on July 6.
Do	17	do	1 part virus to 10,000 of water.	10 mosaic on July 6.
Do	21	do	1 part virus to 100,000 of water.	2 mosaic on July 6.
Do	10	do	Tap water only	All healthy on July 6.
Do	75	do	Not inoculated	Do.
1914.				
April 2	10	Connecticut Broadleaf	Original undiluted virus	6 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	2 mosaic on April 17.
Do	10	do	1 part virus to 10,000 of water	1 mosaic on April 17.
Do	10	do	1 part virus to 100,000 of water.	Do.
Do	20	do	1 part virus to 1,000,000 of water.	All healthy on April 17.
Do	10	Nicotiana rustica	Original undiluted virus	4 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	Do.
Do	10	do	Tap water alone	All healthy.
April 3	10	Connecticut Broadleaf	Original undiluted virus	3 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	5 mosaic on April 17.
Do	10	do	1 part virus to 1,000,000 of water.	All healthy on April 17.
Do	10	do	Tap water alone	Do.
Do	10	Nicotiana rustica	Original undiluted virus	8 mosaic on April 17.
Do	10	do	1 part virus to 1,000 of water.	7 mosaic on April 17.
Do	10	do	do	2 mosaic on April 17.
Do	10	do	Tap water alone	All healthy on April 17.
April 9	10	Connecticut Broadleaf	Original undiluted virus	9 mosaic on April 25.
Do	10	do	1 part virus to 1,000 of water.	7 mosaic on April 25.
Do	10	do	1 part virus to 5,000 of water.	2 mosaic on April 25.
Do	10	do	1 part virus to 10,000 of water.	Do.
Do	10	do	1 part virus to 20,000 of water.	All healthy on April 25.
Do	10	do	1 part virus to 50,000 of water.	1 mosaic on April 25.
Do	10	do	1 part virus to 100,000 of water.	All healthy on April 25.
Do	10	do	1 part virus to 200,000 of water.	Do.
Do	10	do	1 part virus to 500,000 of water.	Do.

TABLE I.—*Effect of dilution upon the infectivity of the virus of the mosaic disease of tobacco*—Continued

Date of inoculation.	Number of plants.	Variety.	Degree of dilution.	Effect.
1914. April 9	10	Connecticut Broadleaf	1 part virus to 1,000,000 of water.	All healthy on April 25.
Do	10	.....do	Tap water alone	Do.
Do	10	.....do	Not inoculated	Do.
Do	10	Maryland Mammoth	Original undiluted virus	9 mosaic on April 25.
Do	10	.....do	1 part virus to 1,000 of water.	6 mosaic on April 25.
Do	10	.....do	1 part virus to 5,000 of water.	5 mosaic on April 25.
Do	10	.....do	1 part virus to 10,000 of water.	2 mosaic on April 25.
Do	10	.....do	1 part virus to 20,000 of water.	All healthy on April 25.
Do	10	.....do	1 part virus to 50,000 of water.	Do.
Do	10	.....do	1 part virus to 100,000 of water.	Do.
Do	10	.....do	1 part virus to 200,000 of water.	Do.
Do	10	.....do	1 part virus to 500,000 of water.	Do.
Do	10	.....do	1 part virus to 1,000,000 of water.	Do.

These tests show beyond question that the virus of the mosaic disease when diluted to 1 part in 1,000 of water is quite as effective in producing infection as the original undiluted virus. A dilution of 1 part in 10,000, however, gives evidence af attenuation. At greater dilutions than this the chances of infection are very greatly reduced. In dilution experiments of this sort, where increasing attenuation of the virus is taking place, it is obvious that no sharp line of demarkation can be found beyond which chances of infection do not exist. Since the solutions are punctured into the leaves with a sharp needle, the quantity of virus taken up and actually introduced into the plant tissues must be exceedingly small, especially for the higher dilutions. It is of interest to consider this fact in connection with the enzymic theory of the mosaic disease, which has been advanced to explain the nature of the disease. This theory assumes that the mosaic disease develops when certain oxidizing enzyms normally present in the plant increase in amount or in activity as a result of various external conditions affecting nutrition and growth.

It is somewhat difficult to reconcile this theory with the fact that a tiny drop of virus diluted to 1 part in 10,000 can readily produce the mosaic disease. It must be assumed that the immeasurably small quantity of oxidizing enzyms carried by this drop is sufficient to increase the normal oxidase content already present in the plant to the extent of a permanent pathological reaction resulting in the mosaic disease. This is highly improbable, since it is well known that the oxidase content of healthy individuals normally varies to a measurable degree in response to various environmental changes.

There seems to be no logical reason for considering that something exists in the constitution of all normal tobacco plants which is always capable of producing the mosaic disease in response to suitable conditions. This conception does not harmonize with the fact that even when the virus of the mosaic disease is highly diluted and the infective substance becomes immeasurably small it is still capable of initiating the disease when introduced into healthy plants. All evidence at hand points to something in the virus quite extraneous to the protoplasmic constitution of healthy plants. Once introduced into the tissues of such plants, this foreign substance rapidly increases in quantity and becomes actively prejudicial to those physiological activities associated with normal nutrition and growth.

In the opinion of Woods and Heintzel this substance constituting the active, pathogenic principle of the virus of the mosiac disease may be regarded as purely chemical, nonliving, enzymic, and a normal constituent of all healthy tobacco plants. According to Hunger, on the other hand, the disease is caused by a toxic ferment not normally present in the cells of healthy plants, but which develops in response to unfavorable conditions of nutrition and growth. These theories are in complete agreement in ascribing to the mosaic disease a spontaneous origin within susceptible plants under favorable conditions. The development of this conception is quite natural if a spontaneous origin is accepted, since this does not admit of a consistent explanation on the basis of parasitism. At the time these theories were evolved, little was known concerning organisms which are smaller than the visible bacteria and yet responsible for parasitic diseases. It was known that the visible bacteria could not pass through the pores of certain filters. It was also discovered that passing the virus of certain diseases through these filters did not necessarily deprive it of its power to infect, although visible parasites were no longer present. At this time this characteristic seemed to remove those diseases connected with a filterable virus from that class of diseases definitely established as bacterial in their origin. Until additional facts had been secured an enzymic origin was perhaps the most plausible explanation for those obscure diseases connected with a filterable virus and supposedly capable of a spontaneous origin within certain plants.

The writer's experiments, however, indicate that the mosaic disease can not be induced to arise spontaneously in healthy plants by the operations of cutting back, repotting, or otherwise subjecting the plants to unfavorable conditions.

SUMMARY

The virus of the mosaic disease when diluted to 1 part in 1,000 of water is quite as effective in producing infection as the original undiluted virus. Attenuation of the virus is indicated in dilutions of 1 part in 10,000 of water. At greater dilutions infection is not likely to occur.

The virus of the mosaic disease is highly infectious to all susceptible, healthy plants. Such plants remain free from this disease so long as all chances of accidental infection are excluded. All evidence at hand indicates that something is present in the virus of the mosaic disease which is extraneous to the protoplasmic organization of healthy plants. This substance greatly increases in quantity when introduced into susceptible plants and interferes with normal nutrition and growth.

Although enzymic activities have been considered responsible for the mosaic disease of tobacco, parasitism, in the writer's opinion, offers by far the simplest and most reasonable explanation of its origin. It may at least be said that the theory of a parasitic origin for the disease more consistently accounts for all the facts at hand than any enzymic conception yet evolved. It seems not only needless but illogical to abandon a simple, direct explanation for one which leads to complexity of thought and yet fails to correlate all the facts at hand.

MOLDINESS IN BUTTER

By CHARLES THOM, *Mycologist*, and R. H. SHAW, *Chemist, Dairy Division, Bureau of Animal Industry*

INTRODUCTION

References to mold in butter are not uncommon in dairy literature, but specific information is lacking as to what forms of mold occur on butter, the conditions which permit mold development, and the actual changes produced in the butter. As met in the market, losses from mold take two forms: (1) The growth of mold upon the tub, lining, or wrapper injures the appearance and salability of the package without seriously affecting the quality of its contents. (2) Mold development upon the butter itself when continued for a considerable period produces changes which can not be eliminated even by the renovation process. Such butter becomes an actual loss. The work reported here aims to cover the biological phases of this problem. The study of the chemical changes produced in the butter will be reported later.

ORIGIN OF BUTTER SAMPLES

Characteristic samples representing the range of conditions and appearances found in commercial butter were obtained through the inspection service of the Dairy Division. These were examined in the mycological laboratory. The number and variety of mold colonies upon each sample were noted and cultures were made to obtain the species represented. The samples were then taken to the chemical laboratory for analysis. Consideration of the known factors influencing mold growth called for the determination of the quantities of water and protein available, together with the percentage of salt as a possible limiting factor.

In Table I are given the analyses of samples of moldy butter from several sources.

TABLE I.—*Analyses of samples of moldy butter*

Sample No.	Water.	Salt.	Curd.[a]	Sample No.	Water	Salt.	Curd.[a]
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
3515..........	11.65	1.98	1.48	3554s..........	7.38	0.63	0.57
3520..........	12.00	.65	1.53	3546a..........	16.02	3.05	.68
3530..........	9.40	2.19	.64	3546b..........	18.00	1.40	.75
3554g..........	11.09	2.66	.66	3546c..........	16.02	3.50	.62
3554r..........	11.72	2.13	.64				

[a] The term "curd," as used in this paper, means the amount of nitrogen multiplied by the factor 6.38.

Samples Nos. 3546a, 3546b, and 3546c were taken from a tub of butter containing three small churnings. The tub had been kept in a refrigerator for three or four months and was very rancid. Since the butter was designed for packing stock to be used in experimental work on renovated butter, no particular care was taken in its manufacture. It happened that the top layer (3546a) and bottom layer (3546c) were heavily salted, while the middle layer (3546b) contained but a small percentage of salt and a considerably higher percentage of water. The top and bottom layers were free from mold; the middle layer showed areas typically representing each of the types of moldiness described in the following pages.

TYPES OF MOLD FOUND IN SAMPLES

From the study of these and other available samples of moldy butter three well-marked types of mold effects are distinguished:

1. SMUDGED, OR ALTERNARIA, TYPE.—In samples Nos. 3515, 3546b, and 3554s, dark, smoky, or rarely greenish colors occurred in patches which suggested soot or dirty-finger marks. Microscopic examination showed mold mycelium with dark-brown or green walls on or under the surface. Frequently these colonies are entirely submerged in the butter. Sometimes hyphæ were observed 4 to 5 mm. below the surface. Spores were rarely found, but colonies transferred to culture media grew freely and fruited normally. The dark-brown or black hyphæ were the most common and proved to be species of Alternaria. Where a greenish color was seen species of Cladosporium developed. These submerged areas suggest the appearances noted by Patterson (1900),[1] and attributed to *Stemphylium butyri* Patterson. The occurrence of Cladosporium in butter has been studied by Jensen (1900) and the organism found was named by him "*Cladosporium butyri.*" The species of Cladosporium, however, are abundant upon all kinds of roughage fed to cows, and the spores find entrance to the milk from the handling of such feed by the milkers. One of the writers had access to cultures made from many samples of cream by the bacteriologists of the Storrs Agricultural Experiment Station some years ago. In these cultures colonies of Cladosporium were so abundant as to indicate that spores of these species remain with the cream after separation. Species of Alternaria are very common in the same circumstances and appeared in these cultures, but less abundantly. Their appearance as colonies in the butter, therefore, is due to the ability of these species to grow in the very severe conditions imposed by a mass of butter. One of the writers has found a species of Alternaria growing and fruiting in a box of shoe paste. Species of this group have also been isolated from various forms of fat when small inclusions of water occur. Few other graminicolous fungi seem able to produce colonies under these conditions, though spores of many kinds are undoubtedly present.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 310.

In at least one sample, contributed by Dr. G. P. Clinton, of the Connecticut Agricultural Experiment Station, and again in a sample of fat studied by Dr. C. N. McBryde, of the Bureau of Animal Industry, a fungus producing abundant orange to red mycelium and red blotches upon the butter was obtained. In butter and in the culture media used no spores have thus far been obtained. This organism grows under the same conditions as Alternaria.

2. GREEN-MOLD TYPE.—Green molds were found more or less frequently upon all the samples tabulated except Nos. 3546a and 3546c. Cultures of these molds proved to be species of Penicillium. Three common species were often found. These were *P. roqueforti*, a variety or strain of *P. expansum*, and *P. chrysogenum*. Several other forms difficult to identify were occasionally obtained. Aside from *P. roqueforti*, these are identifiable only by careful culture and comparison. These molds form green patches on the surface and follow seams or cracks into the mass. In one tub (No. 3515), where extensive moldy areas were found in cracks and seams, the presence of *P. roqueforti* was suggested by a strong odor and flavor resembling that of Roquefort cheese. Marked physical changes in the fat itself were noticeable. Culture confirmed the identification of the organism. So far as observed, no such extensive changes were produced by the other species. The storage of butter in tubs is accompanied by low percentages of free oxygen[1] in the butter suggestive of the conditions in Roquefort cheese (Thom and Currie, 1913). Mold is found upon the liners, upon the inside of the tub itself, and in the cracks of the butter. In all these places interchange of gases is very slow, thus favoring the dominance of Roquefort mold, which is more tolerant of such conditions than other species.

3. OIDIUM TYPE.—The third form produces various shades of orange-yellow discoloration, with little or no surface growth. Culture and microscopic examination show that these areas are produced by *Oidium lactis*. This organism grows to the depth of several millimeters within the mass of butter as a complex mycelium with hyphæ varying in diameter with the size of the spaces between the masses of fat. Some spores are formed and at times surface-fruiting areas. Bacterial activity is commonly associated with the presence of this mold.

BLACK MOLDS, OR MUCORS.—Where butter has been moist enough for loose masses of surface mycelium to grow, mucors are sometimes seen. These molds are found by culture to be present in many other samples in which no visible colonies are produced.

EXPERIMENTAL WORK

To study the conditions favorable to mold growth in butter special samples of butter were prepared, some low in water content and some high in water content, some thoroughly washed to reduce the curd con-

[1] Unpublished results of Dr. D. C. Dyer, of the Dairy Division.

tent and some with casein added to raise the protein content. One ounce of salt to 1 pound of butter was used in some samples; no salt in others. Slices of butter of each kind were put into Petri dishes and inoculated with a series of molds obtained from butter. Among these were *Oidium lactis*, *Mucor* sp., *Alternaria* sp., and several species of Penicillium. The dishes were then allowed to stand in an incubator at the temperature and relative humidity of the laboratory for several days. Absolutely no surface growth of mold was obtained. Part of these Petri dishes were then placed in moist chambers and it was found that mold colonies developed upon every sample so placed. These growths included not only the species inoculated into the butter but other forms whose spores were present in the butter as made. At the low relative humidities prevailing in the laboratories of the Dairy Division from February to April, 1914, no mold colonies were able to develop in butter representing a range in water content greater than the usual range of percentage in market butter.

The addition of 2 to 3 per cent of water to butter containing but 14 or 15 per cent does not make the quantity of water present sufficient to support mold growth aside from conditions of high humidity.

RELATION OF HUMIDITY TO MOLD GROWTH

In moist-chamber culture comparison between samples containing normal and low protein with samples containing excess or added protein showed that mold growth was more rapid and extensive when protein was added. The failure of molds to grow in these same cultures under the ordinary humidity conditions of the laboratory proved that the essential factor in molding was not protein, but water.

To define these humidity relations more closely, three desiccators were prepared in which definite relative humidities could be maintained. For this purpose the bases of the desiccators were filled with sulphuric acid standardized to the specific gravities—from Hastings's (1909) table—required to maintain, respectively, 90 per cent, 79.6 per cent, and 69.6 per cent relative humidity. Three samples were used: One sample of butter was made with low-salt content (0.55 per cent); one at normal salting (2.43 per cent); and one sample of butter fat, free from water, with skim-milk powder added.

The composition of these three samples is given in Table II.

TABLE II.—*Composition of samples of butter used in mold-growth tests*

Character of sample.	Water.	Salt.	Curd.
	Per cent.	*Per cent.*	*Per cent.*
Normal-salt butter	15.2	2.43	0.62
Low-salt butter	15.6	.55	.62
Butter fat + dry skim milk	None.	None.	.48

Three slices, one from each of these samples, were put into each one of a series of 24 Petri dishes. The three slices in each dish were thus under absolutely the same conditions. Six species of mold were then selected and were heavily inoculated into the plated slices—each slice in four Petri dishes being inoculated with one species of mold. Four sets of six dishes each were thus available. One set of six Petri dishes was put into a moist chamber (approximately 100 per cent of relative humidity), and one each into the desiccators at 90, 79.6, and 69.6 per cent relative humidity. The cultural results are given in Table III.

TABLE III.—*Effects of salt and humidity on mold growth in butter*[a]

Mold.	Growth in moist chamber.			Growth under relative humidities of—								
				90.6 per cent.			79.6 per cent.			69.6 per cent.		
	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.	Salted butter.		Butter fat and curd.
	0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.		0.55 per cent.	2.43 per cent.	
Alternaria sp.	0.9	0	0	0.3	0	0	0.6	0	0	0	0	0
Mucor sp.	(?)	0	0	0	0	0	0	0	0	0	0	0
Oidium sp.	[b] 0.6		0	.5	0	0	.5	0	0	.3	0	0
Penicillium roqueforti	.5	0	0	.5	.2	0	0(?)	0	0	0(?)	0	0
Penicillium chrysogenum	.5	.1	.8	.4	.2	.3	.3	0	.2	.3	0	0
Penicillium expansum	.5	.1(?)	0	.3	0	0	.3	0	0	0	0	0

[a] A typical colony would be designated as 1.0; lesser growths by decimal fractions.
[b] Submerged.

Examination of this table shows that a single species, *Penicillium chrysogenum*, was able to produce a colony upon the butter fat plus the water obtainable from the air. Careful examination of the other samples showed no mold. In the low-salted butter, however, with 15.6 per cent of water marked growth occurs; the water in this butter is therefore to be regarded as an essential factor in the molding found here. The butter containing 2.43 per cent of salt shows determinable growth from but two species, *P. roqueforti* and *P. chrysogenum*. No growth of species of Alternaria, Oidium, or Mucor was found upon this butter. The low-salted butter shows very appreciable mold colonies of all species except the Mucor. Growth was greatest in the moist chamber. Nearly as good growth was obtained, however, with a relative humidity of 90.6 per cent, and considerable growth in four of the species with 79.6 per cent. In the presence of 69.6 per cent there was very little visible mold, even in the low-salted sample. The individual samples of low-salted butter all showed the characteristic orange-yellow colors due to the development of spores of *Oidium lactis*, which were evidently present from the first in all slices. In this experiment the organism grew only in its submerged form; hence, it was little affected by the relative humidity to which the other species responded so clearly. Alternaria and Oidium developed

only in the low-salted samples. Alternaria appeared in several places without inoculation. *Rhizopus nigricans* was found once in a moist-chamber sample. *Aspergillus fumigatus* and *A. niger* both appeared in one or more cases, but none of these species appeared where the percentage of salt was 2.43.

The same fact is illustrated on a larger scale by the tub of butter analyzed as No. 3546 in Table I. Of the three samples packed together in one tub the middle layer was low-salted and typically moldy, while the top and bottom layers were free from mold.

These results harmonize fully with the data from the analysis of butter as found in Table I and with the preliminary cultural data as given in subsequent experiments. Two of the three types of moldiness, the smudged and the orange-yellow forms, occur only in butter containing less than 2 per cent of salt. Even with green molds under high humidities and at temperatures far above those used in storage, growth in these experiments was negligible in butter with a salt content of 2.43 per cent.

THE SALT FACTOR IN MOLD GROWTH

The salt factor in butter is calculated as follows: Thompson, Shaw, and Norton (1912), in analyzing 695 samples of American creamery butter, found an average water content of 13.9 per cent; salt content, 2.51 per cent; and curd content, 1.18 per cent. This amount of salt in solution in the water present forms, therefore, approximately a 13 per cent brine, which represents the brine formed by adding 18 parts of salt to 100 parts of water. If the same water content be assumed and the salt content found be 1 per cent, the brine present is 5.1 per cent (made by adding 7.1 parts of salt to 100 parts of water); with a salt content of 2 per cent, this strength would be 10.2 per cent; with 3, 15.3 per cent; and with 4, 20.4 per cent. For purposes of mold growth the strength of the brine found is one very significant factor. Another factor is represented by the distribution of air and moisture throughout the mass of butter, and still a third by the relative humidities to which the butter is subjected.

To obtain more complete cultural data for comparison, a series of cultures was made with media containing known percentages of salt. For this purpose 6.5 per cent of salt was introduced into one lot of Czapek's agar (Dox, 1910) and 14 per cent in a second lot. The first represents approximately the proportion of brine in butter with 1.3 per cent of salt, the second the brine with a content of about 2.8 per cent of salt. These cultures were grown in a moist chamber to eliminate the concentration of the brine by drying. The cultural results are given in Table IV.

TABLE IV.—*Cultural results with media containing known percentages of salt*[a]

Mold.	Percentage of salt. 6.5	Percentage of salt. 14.4	Mold.	Percentage of salt. 6.5	Percentage of salt. 14.4
Alternaria sp. 3515	0.7	0.2	Penicillium roqueforti 3515C	1.0	[b] 1.0
Alternaria sp. 3513	.7	.2	Penicillium expansum	.9	.4
Alternaria sp. 3546	.7	.2	Penicillium stoloniferum, var	1.0	[b] .7
Cunninghamiella sp.	.6	0	Penicillium chrysogenum	.9	.7
Fusarium sp.	.4	0	Penicillium purpurogenum	......	0
Mucor sp. 3513	.7	0	Red mold 3536.3	.3	0
Mucor sp. 3514D4	.6	0	Rhizopus nigricans	.7	0
Mucor sp. 3514C1	.6	0	Trichoderma sp	.3	0
Mucor sp. 3532	.5	0			
Oidium lactis	.1	0			
Penicillium sp. 3529a	1.0	[b] 1.0			
Penicillium roqueforti	.9	[b] .4			

[a] A typical colony is designated as 1.0; lesser growth by decimal fractions.
[b] These cultures developed slowly, but finally reached the condition indicated.

These cultural results agree with other data published recently (Thom, 1914). Two more series of cultures were made, containing approximately 18 and 21 per cent of salt. In these such organisms as produced marked growth with a salt content of 14.4 per cent were carried, together with other species of Penicillium and Aspergillus. *Penicillium chrysogenum, P. stoloniferum, Penicillium* sp. 3529a and *Aspergillus repens* produced considerable growth with 18 per cent of salt. Three other organisms produced slight growth. With 21 per cent of salt no colonies were obtained, although spores of *P. chrysogenum* germinated. Comparison with the results of butter inoculation shows that Czapek's solution sustained much larger growth than butter containing comparable percentages of salt. To show the results of these culture series for the organisms obtained from butter, the graphic representation (fig. 1) was prepared. The four series reported were calculated as representing approximately brine conditions in butter containing 1.3, 2.7, 3.4, and 4.1 per cent of salt. Even under the very favorable conditions offered by the culture media, temperature, and humidity used, the mold growth found in the second series was small and in the third series was negligible.

DISCUSSION OF RESULTS

From the data already given, mold is seen to attack the butter itself if unsalted or very lightly salted. Normally salted butter may be affected by green mold only if held under conditions very favorable to mold growth. In general such losses are not great. Both the species of Oidium with its orange-yellow patches and the smudges of Alternaria disappear promptly when even very moderate salting is practiced. These are the important factors in losses of unsalted butter as studied by Jensen (1901 and 1908). Since *Oidium* sp. penetrates the mass of butter

and produces marked discoloration, as well as bad flavors, salting, if practiced at all, should be heavy enough to eliminate this group of organisms. Green molds may damage normally salted butter if cracks and open spaces are left by bad packing. In most cases such mold will be confined to liners and containers if the packing is fairly well done. Rogers (1906) found that paraffining the tubs or boxes used prevented mold on both container and liner. The paraffin prevented the escape of water which would leave the air spaces necessary for mold growth, thus

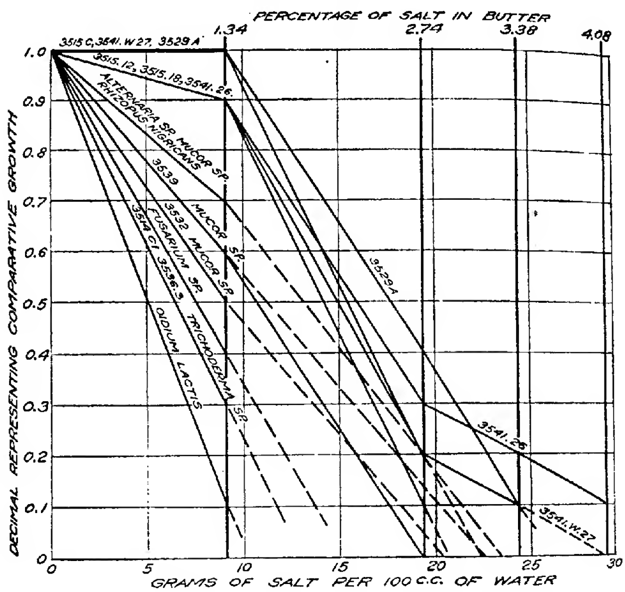


FIG. 1.—Graph showing the effect of salt on molding. Cultural results with organisms obtained from butter. Nos. 3515C, 3541W27, 3529A, 3515.12, 3515.18, and 3541.26 were species of Penicillium. No. 3514C1 was a species of Mucor and No. 3536.3 was the sterile red mold from butter. The dotted portions of the graph represent hypothetical courses for organisms disappearing at percentages not determined but limited by the next experiment.

preventing also loss of weight from the butter itself. Previous papers have taken no account of the presence of mold spores in the butter itself. All possible treatment of containers will fail unless conditions are produced which will prevent the growth of these spores. The same conditions which stop the growth of molds present on the paper and wood of the package also prevent the spores in the butter from growing.

In all storage of butter the temperature factor must not be neglected. Mold growth is progressively reduced by low temperatures. Work elsewhere reported shows that species of Penicillium (Thom, 1910, p. 92, 93, 105) grow very slowly as the temperature approaches the freezing point.

If, as in butter, the fluid present is a strong brine, the temperature must be actually carried considerably below the freezing point of water to eliminate danger from the growth of micro-organisms. Temperatures a few degrees above freezing accompanied, as they frequently are, by moist conditions are favorable to molding in butter. Unsalted butter is more subject to deterioration from micro-organisms than salted butter. Successful storage of such butter is therefore even more dependent upon scrupulously clean dry refrigeration at low temperatures than is the case with salted butter. Cellars and ice refrigeration rarely furnish conditions which will prevent mold growth in unsalted or low-salted butter, although such growth may be delayed or reduced. Butter properly made and salted normally, as indicated above, will not show mold under reasonably careful handling.

SUMMARY

(1) Mold in butter usually takes three forms:

a. Orange-yellow areas with a submerged growth of mycelium are produced by *Oidium lactis*.

b. Smudged or dirty-green areas either entirely submerged or with some surface growth are produced by species of Alternaria and Cladosporium.

c. Green surface colonies are produced by species of Penicillium, or, more rarely, Aspergillus, either upon the butter, causing decomposition, or upon the container or wrappings, injuring the appearance of the sample in the market.

(2) Species of Oidium, Alternaria, and Cladosporium can not develop in butter containing 2.5 per cent of salt. The occurrence of any of these forms in a sample of butter indicates low salting.

(3) Excess of curd favors mold growth. Well-washed butter is less subject to mold.

(4) Leaky butter—butter from which water of buttermilk exudes and collects in the wrappings or in the container—furnishes the best conditions for the beginning of mold growth. From these wet areas colonies may spread to the butter itself.

(5) Wet surfaces, wet wrappings, or high humidity are essential to mold growth in butter. Mold will not grow upon the surface of a piece of butter exposed to humidities of 70 per cent or lower. The water in the butter is thus not sufficiently available to the mold to support the development of a colony, unless evaporation is reduced by high humidities. In closed packages, wet or damp cellars, or carelessly packed masses with cracks or fissures in which moisture collects, mold may seriously injure the appearance of butter packages or actually induce great changes in the butter itself.

(6) Salt up to 2.5 to 3 per cent in butter is sufficient to eliminate mold or reduce it to negligible amount. This is equivalent to the use of a 12 to 15 per cent brine.

LITERATURE CITED

DOX, A. W.

1910. Intracellular enzyms of Penicillium and Aspergillus, with special reference to those of Penicillium camemberti. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 120, p. 37.

HASTINGS, M. M.

1909. Cold-storage evaporimeter. U. S. Dept. Agr., Bur. Anim. Indus. Circ. 149, p. 6.

JENSEN, ORLA.

1901. Studien über das Ranzigwerden der Butter. In Landw. Jahrb. Schweiz, v. 15, p. 337.

PATTERSON, FLORA W.

1900. New species of fungi. *In* Bul. Torrey Bot. Club, v. 27, no. 5, p. 285.

ROGERS, L. A.

1906. Investigations in the manufacture and storage of butter. II.—Preventing molds in butter tubs. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 89, 7 p., 1 fig.

1908. Paraffining butter tubs. U. S. Dept. Agr., Bur. Anim. Indus. Circ. 130, 6 p., 1 fig.

THOM, CHARLES.

1910. Cultural studies of species of Penicillium. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 118, p. 92, 93, 105.

1914. The salt factor in mold-ripened cheeses. *In* Storrs Agr. Exp. Sta. Bul. 79, p. 387–394.

——— and CURRIE, J. N.

1913. The dominance of Roquefort mold in cheese. *In* Jour. Biol. Chem., v. 15, no. 2, p. 249–258.

THOMPSON, S. C., SHAW, R. H., and NORTON, R. P.

1912. The normal composition of American creamery butter. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 149, p. 10.

SUSCEPTIBILITY OF CITROUS FRUITS TO THE ATTACK OF THE MEDITERRANEAN FRUIT FLY

By E. A. BACK, *Entomological Assistant*, and C. E. PEMBERTON, *Scientific Assistant*, *Mediterranean Fruit-Fly Investigations, Bureau of Entomology*

INTRODUCTION

Since the discovery in 1910 that the Mediterranean fruit fly (*Ceratitis capitata* Wied.) had become established in the Hawaiian Islands, the fruit growers, and especially the citrous fruit growers, of the mainland States have increasingly feared that this dreaded pest would be able to gain access to the mainland on some one of the many ships plying between Honolulu and the Pacific coast and would appear in the citrous orchards of California and Florida. In addition to this danger from the Pacific, there have been similar fears regarding imported fruits from the Bermudas and the Mediterranean regions. While investigations carried on by the Federal Horticultural Board have shown that the opportunity for entry and establishment of the fly from these trans-Atlantic countries is very slight, there remains the ever-present danger that sooner or later this pest will reach the mainland from the Pacific, in spite of the increasingly rigid quarantine of Hawaiian host fruits. It is therefore opportune to record data secured in the Hawaiian Islands which tend to show that even if this fruit fly should obtain a foothold in the warmer portions of the United States, it probably would not be the serious pest to citrous fruits that previously published literature would indicate.

HISTORICAL REVIEW

This literature has been full of references to the havoc caused to citrous fruits by the Mediterranean fruit fly. The first published reference is by Latreille, who states (1817)[1] on the authority of Cattoire that the colonists of Mauritius could with difficulty obtain citrous fruits sound at maturity, on account of the attacks of a dipterous insect that deposited eggs in the fruit. MacLeay (1829) writes of this pest as an insect very destructive to oranges and states that fully one-third of the oranges arriving in London from the Azores were in a decayed condition as a result of the attacks of this pest. He also secured the insect from citrous fruits in Madeira and the Cape Verde Islands. F. DeBreme (1842) speaks of this fruit fly as a pest to oranges near Malaga, Spain; and Westwood (1848), under the caption "The Orange Fly," mentions securing specimens from

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 330.

Journal of Agricultural Research, Dept. of Agriculture, Washington, D. C.

Vol. III, No. 4
Jan. 15, 1915
K—13

decayed oranges received at London from St. Michael. Villeneuve (1859) exhibited before the Entomological Society of France an infested orange from Algeria, and Laboulbène (1871) describes the injuries caused by the fruit fly to oranges in Algeria and quotes from notes furnished him by Boisduval to the effect that at Bildah and in all Algeria the orange crop was completely destroyed by the insect. On the other hand, Rondani (1870) writes that the species is rare in Spain and is found in Italy only in the southern part.

While the purpose of this article is not to record the literature of this fruit fly, these few references are sufficient to show that much of the early literature greatly emphasizes the destructiveness of the Mediterranean fruit fly to citrous fruits and has laid little stress upon other fruits more susceptible to attack. It is also interesting to note that much of this older literature, which has been generously copied by later writers, records damage to citrous crops grown in very equable climates and in localities where presumably, as in the Hawaiian Islands, there are many host fruits whose commercial value was so small that they escaped the notice of these writers, who judged of the seriousness of the pest by the fruits arriving at their home markets or from common reports. It is also very possible, with our more exact knowledge of the causes of the decay of fruit in transit and of the wholesale shedding of citrous fruits in the field, due to several fungous diseases, to question the reliability of some of the earlier statements.

HOST FRUITS

Apparently the first observer who did not entirely agree with MacLeay's statement that whenever a puncture is found in the rind of the orange "there is a worm concealed in the interior" is Laboulbène, who said that when he compared his observations on the damage done to oranges by the Mediterranean fruit fly with those recorded by others he found certain contradictory facts which needed further investigation. These contradictory facts, although Laboulbène did not know it, were concerned with what has been determined by the writers as an excessive mortality occurring among the eggs and larvæ of the Mediterranean fruit fly in the orange rind. This mortality, which in the examination of 39 grapefruit that were yellow in color amounted to 99.7 per cent of 7,722 forms, as shown in Table I, will prove a very effective factor in checking this pest in the citrous regions of the United States, especially when combined with the climatic and floral characteristics of these citrous regions and the method of growing and harvesting the fruit.

TABLE I.—*Results of examinations of ripe citrous fruits infested by the Mediterranean fruit fly*[a]

Kind of fruit.	Number of fruits examined.	Punctures.		Eggs.		Larvæ.						Total number of forms examined.
						Alive.			Dead.			
		Empty.	Not empty.	Normal.	Abnormal.	In puncture.	In rag.	In pulp.	In puncture.	In rag.	In pulp.	
Grapefruit	39	123	378	959	5,882	20	1	0	534	326	0	7,722
Lemon	50	380	185	693	729	29	0	0	339	15	0	1,805
Lime	50	180	218	345	187	0	0	25	474	424	0	1,455
Shaddock No. 1	14	0	44	5	36	0	152	55	17	38	0	303
Shaddock No. 2	14	48	237	6	38	5	2	3	405	696	0	1,155
Kusaie lime	17	194	80	196	201	0	5	0	156	280	0	838
Sweet orange	58	251	452	287	397	55	0	17	1,237	1,652	0	3,645
Sour orange	28	57	174	664	1,026	20	9	231	347	59	1	2,357
Chinese orange	85	1	115	207	286	0	8	383	8	14	17	923

[a] These examinations were made sufficiently long after the fruits were gathered to permit all eggs to hatch. All eggs recorded in tables are in reality dead, even though certain of them are marked "normal" in appearance.

The excessive mortality referred to does not mean that citrous fruits are less attractive to the adult Mediterranean fruit flies or that the fruit of certain species of the Citrus family is not capable of becoming badly infested. Reference to Table I shows that the female fly freely oviposits in grapefruit, lemons, limes, shaddocks, and sweet, sour, and Chinese oranges. Whatever may be the degree of preference shown by the females for other fruits, it is not great enough, at least under Hawaiian conditions, to lead them entirely to ignore citrous fruits, even when these are grown in close proximity to such a favored host fruit as the peach. A study of the data in Table II shows that the female has a much stronger preference for the mango (*Mangifera indica*) and the ball kamani (*Calophyllum inophyllum*) than she has for the orange or lemon. While the data are very limited as to the amount and the number of fruits treated, they are indicative of conditions in the field covering a larger range of fruits. In Bermuda during December, 1913, the senior writer found oranges unaffected while Thevetia and loquats (*Eriobotrya japonica*) were well infested. Unfortunately for experimental purposes there are in Hawaii no large Citrus orchards free from other host fruits. Instead there are growing a great profusion of host fruits, chiefly in city or suburban districts, which furnish a rapid succession of fruit flies. No matter, therefore, what preference the ovipositing females may show for noncitrous fruits, the flies are present in such large and constantly augmented numbers that the slowly maturing citrous fruits are bound to be attacked. This is especially true during the months of December, January, and February, when a comparatively small number of host fruits other than Citrus are in season. Like conditions also exist at other seasons of the year during the short intervals between the ripening of other host fruits. While many of these host fruits ripen quickly, the citrous fruits, with the exception of the Chinese orange, develop

slowly and offer themselves for attack over a considerable length of time. Female fruit flies have been seen in Honolulu ovipositing in certain grapefruits and oranges over a period of two or three months. It is not therefore contradictory to the statement that citrous fruits are not the preferred hosts of the fly that we find so large a number of punctures recorded in Table I.

TABLE II.—*Host-fruit preference of the Mediterranean fruit fly*

Experiment No.	Combination and condition of fruits.	Number of punctures.	Number of eggs.
1	Orange, ripe but green in color	5	17
	Mango, partially ripe	10	92
2	Orange, partially ripe	6	22
	Mango, partially ripe	18	101
3	Orange, ripe	16	105
	Mango, ripe	15	135
4	Orange, partially ripe	9	23
	Mango, partially ripe	8	84
5	Orange, ripe	0	0
	Mango, ripe	2	10
6	Lemon, green in color	0	0
	Mango, partially ripe	5	53
	Ball kamani, partially ripe	0	0
7	Lemon, ripe	1	0
	Mango, ripe	4	58
	Ball kamani, ripe	3	114
8	Lemon, partially ripe	0	0
	Mango, partially ripe	8	54
	Ball kamani, partially ripe	4	376
9	Mango, nearly ripe	3	15
	Ball kamani, ripe but sound	0	0
	Lemon, beginning to turn color	0	0
10	Orange, ripe	1	10
	Mango, partially ripe	1	28
	Ball kamani, partially ripe	4	242
	Rose-apple (*Caryophyllus jambos*) nearly ripe	4	24
11	Orange, ripe	1	3
	Rose-apple, ripe	2	11
	Mango, ripe	11	126
12	Lemon, beginning to turn yellow	0	0
	Mango, partially ripe	3	15
	Ball kamani, mature but solid	0	0

HABITS OF MEDITERRANEAN FRUIT FLY

For those unfamiliar with the Mediterranean fruit fly it may be briefly stated that this pest belongs to the order Diptera and the family Trypetidae. It is one of many species of this family that cause much injury by their attack upon various fruits. In the Hawaiian Islands the fly attacks over 30 different species of fruits and has caused great financial loss. The adult female, which is about the size of the ordinary house fly, pierces the skin of the host fruit and forms an egg cavity beneath, in which she deposits eggs. The larvæ which hatch from these eggs either burrow at once to the center of the fruit, as in the peach (*Amygdalus persica*), or may feed in the outer portion, as in the star-apple (*Chrysophyllum*

cainito). In either case the fruit is rendered worthless by the developing larvæ before it is ripe. When the larvæ become well grown, they leave the fruit (Pl. XL) either before or after it has fallen and enter the ground or other protected places and transform to the pupal stage, from which the adult later emerges. In Hawaii the Mediterranean fruit fly requires in passing from the egg to the adult stage from 14½ days in summer to about 47 days during the coldest winter weather. In this paper the words "puncture" and "egg cavity" are often used synonymously.

PROPORTION OF EGG PUNCTURES CONTAINING EGGS

The data in Table I show that many of the punctures in the rind made by the female contain no eggs. In one of the most favored host fruits, the peach, practically all the punctures made contain eggs. Of 534 punctures made in 112 peaches but 13 were empty. The rind of lemon contains a much higher percentage of empty punctures than that of any of the other citrous fruits in Hawaii, except the Kusaie lime (*Citrus limetta*). In the 50 fruits examined 380 empty punctures were found, as compared with 185 with eggs. Practically all punctures in Chinese oranges contain eggs. (See Table I.) In the 85 fruits examined only 1 puncture out of 116 was empty. Grapefruit, or pomelos, shaddocks, and sour oranges seem to be preferred for oviposition to the ordinary budded or seedling oranges. It has been noted that adult fruit flies, especially the males, congregate in large numbers on citrous trees, and in the laboratory both sexes are quickly attracted to pieces of cut rind of citrous fruits. They seem to take pleasure in feeding upon the oils and other substances contained in the broken cells, and it is possible that in the field their liking for juices made available by the process of forming the egg cavity is so great that the females discontinue ovipositing and begin feeding. The large percentage of empty punctures in lemons and Kusaie limes, in particular, can not be ascribed to a lack of ripeness, as in practically all instances the fruits examined were fully grown and a large percentage were colored and overripe.

MORTALITY OF EGGS AND LARVÆ

Although many punctures in citrous fruits may be empty, others contain a sufficient number of eggs to infest badly a fruit not so well equipped by nature to withstand attack. Out of 13 punctures in one grapefruit 9 contained 76, 153, 32, 25, 18, 8, 46, 113, and 9 eggs, respectively. While this is a larger number of eggs than is usually found in a like number of punctures, it is sufficient when supplemented by the data from other citrous fruits to arouse interest in finding a reason why, with so many eggs deposited in citrous fruits, so very few flies succeed in reaching maturity. (See Table I.) Thirty-nine oranges, either yellow or orange in color, picked from the trees on September 13, 1913, and containing an average of 32 punctures, with a maximum of 108 and a minimum of 7

punctures, developed no flies, and their pulp was in a sound though somewhat shrunken condition after they had been held in the laboratory for one month.

That there takes place in citrous fruits a very great and previously unrecorded mortality among the eggs and larvæ is clearly set forth in the data in Table I. This mortality is especially pronounced in grapefruit, lemons, sweet oranges, and Kusaie limes in Hawaii, is less in Hawaiian limes and sour oranges, and very much less in Chinese oranges. It has been a common belief among many in Hawaii that citrous fruits are too acid to permit the larvæ to live in their pulp until ripe, in spite of the contradictory evidence that the quite acid Chinese orange is generally infested. The data in Table III are here given in proof that no citrous fruit, not even the lemon, is too acid for the development of Mediterranean fruit-fly larvæ. A study of the data shows that there is a high mortality among larvæ transferred to citrous fruits. Too much importance, however, should not be placed upon this, as these fruits must be mutilated somewhat in the process of transferring the larvæ and therefore are more easily attacked by decay fungi, which bring about a condition not especially desirable for the growth of larvæ and often positively fatal to their development. The data are of special interest in proving that even first-instar larvæ are able to reach maturity in well-grown though green lemons. The percentage of first-instar larvæ maturing in green lemons was in several instances even greater than that of larvæ maturing in ripe lemons.

TABLE III.—*Development of larvæ of Mediterranean fruit fly in citrous fruits*

Transference of larvæ.			Instar.	Number of larvæ.		
From—	To—	Date.		Transferred.	Died.	Matured.
Ball kamani	Ripe lemon	Feb. 19	First	41	23	18
Winged kamani (*Terminalia catappa*).	do	12	do	120	120	0
Chinese orange	Green lemon	28	do	17	17	0
Apple	California lemon	Mar. 12 to 15	do	480	381	99
Ball kamani	Ripe lemon	Feb. 20	Second	160	117	43
Do	Green lemon	20	do	20	17	3
Winged kamani	Ripe lemon	12 to 17	do	120	110	10
Chinese orange	do	14 to 18	do	40	32	8
Do	Green lemon	18 and 19	do	40	26	14
Ball kamani	Ripe lemon	18	Third	60	33	27
Winged kamani	Green lemon	16	do	80	50	30
Do	Ripe lemon	12 to 16	do	260	207	53
Chinese orange	Green lemon	18	do	60	48	12
Do	Ripe lemon	18	do	220	125	95
Ball kamani	California grapefruit	20 and 21	First	80	71	9
Winged kamani	do	21	do	20	13	7
Do	do	13 to 21	Second	120	67	53
Ball kamani	do	20 to 22	do	240	137	103
Do	do	20 and 21	Third	60	27	33
Winged kamani	do	21	do	60	22	38
Papaya	Ripe sweet orange	16	First	20	12	8
Chinese orange	do	20	do	20	20	0
Winged kamani	do	12	do	40	16	24
Do	do	12 and 13	Second	140	65	75
Papaya (*Carica papaya*)	do	17	do	40	28	12
Chinese orange	do	16	do	60	32	28
Winged kamani	do	13 to 16	Third	120	24	96
Papaya	do	17	do	40	20	20
Chinese orange	do	16	do	90	39	51
Ball kamani	do	12	do	40	8	32

The data in the tables make it evident that the cause of the mortality is not the acidity of the fruit. The figures are of interest in showing that the mortality occurs largely in the rind, either among the eggs in the punctures or among the newly hatched larvæ in the egg cavity where they hatch or in the rag beneath. The percentages of mortality occurring among eggs and newly hatched larvæ are given in Table IV.

TABLE IV.—*Mortality of eggs and larvæ of Mediterranean fruit fly in the rind*

Kind of fruit.	Total number of forms examined.	Mortality in percentages in rind.		
		Among eggs.	Among larvæ.	Total.
Grapefruit	8,222	90.5	9.3	99.8
Lemons	991	76.1	21.0	97.1
Limes	1,054	43.1	55.6	98.7
Kusaie limes	838	47.6	51.8	99.4
Sweet oranges	3,635	19.0	79.0	98.0
Sour oranges	2,357	71.7	17.3	89.0
Shaddock No. 1	1,155	3.8	95.3	99.1
Shaddock No. 2	303	13.5	18.1	31.6
Chinese orange	1,039	3.7	46.5	51.2

MORTALITY AMONG EGGS

As eggs deposited in such host fruits as the peach and loquat hatch with great certainty, the writers were of the opinion that the oil in the oil cells of the rind was an active agent in killing the eggs in citrous fruits. In puncturing the rind in the process of forming the egg cavity the female is likely to drill through one or several oil cells and the oil thus freed, though not of sufficient quantity to drive the female away, is sufficient in many instances to kill all or many of the eggs deposited. The data in Table V indicate that there is no question that the oil causes the death of the eggs. Only 163 out of 1,600 eggs treated with oil hatched, as compared with 1,313 out of 1,600 eggs held as a check. The eggs under observation were dissected out of punctures in California apples and placed on fresh foliage in moist jars. The treated eggs were not sprayed according to the usual method, but by bending over them a portion of the rind of fresh orange (in the first record) or fresh lemon (in the second and third records) so that the oil from the ruptured cells reached the eggs in that fine mistlike spray familiar to all who have eaten freshly gathered oranges. The much larger number of treated eggs that hatched in the last record is accounted for by the writers by their being fully 20 hours older than those in the second lot when treated. It should be stated that eggs removed from their host do not usually all hatch, as some sustain slight injuries and others may be infertile. See Table V.

TABLE V.—*Effect of oil from rind of orange and lemon upon the hatching of eggs of the Mediterranean fruit fly*

Period of depositing eggs.	Treated with oil.	Check.	Number of eggs hatched.	
			Treated.	Check.
1.30 p. m., Mar. 22, to 9 a. m., Mar. 23	800	800	41	609
9 a. m. to 1 p. m., Mar. 27	400	400	3	342
9 a. m., Mar. 27, to 9 a. m., Mar. 28	400	400	119	364
Total	1,600	1,600	163	1,313

Further evidence that the oil in the ruptured cells is the killing agent is the very small mortality among the eggs deposited in the Chinese oranges. Since in this fruit the rind is only about two twenty-fifths of an inch in thickness, the female is compelled to deposit her eggs either through the rind into the pulp or in a position between and parallel to the rind and pulp, but at a distance from the puncture that seems to be a protection from any oil set free by the puncturing process. Of 609 eggs thus deposited between the rind and the pulp 600, or 98.5 per cent, hatched, as determined by an examination of 85 fruits one week after they had been picked. A comparison of the 98.5 per cent hatched in Chinese oranges with the percentage of the mortality among eggs in other citrous fruits emphasizes the part the oil has in causing mortality among eggs. It is also interesting to note in passing that the eggs in Kusaie limes, the rind of which is sufficiently thick so that the eggs are deposited directly beneath the puncture, die with great regularity, while the eggs in Hawaiian limes, the rind of which may be sufficiently thin to permit the eggs being deposited as in Chinese oranges or so thick (according to the individual tree) that the eggs are laid either in the cavity in the rind or between the rind and pulp but directly beneath the puncture, suffer a degree of mortality between that of eggs deposited in Chinese oranges and Kusaie limes.

While in Chinese oranges the eggs deposited between and parallel to the rind and pulp hatch with great regularity, those deposited through the rind into the pulp are subjected to a mortality caused either by excessive moisture or lack of air. Eggs thus laid are usually placed beneath the skin covering the pulp, and the fascicle which they compose appears, after the rind has been removed, as a dull white spot that is easily overlooked. Usually no trace of the opening through the skin covering the pulp through which the eggs have been deposited can be found. The eggs appear thoroughly sealed within the pulp. In some few instances the opening is distinct and occasionally an egg is left in it, half in and half out of the pulp. When these openings in the skin occur, the eggs appear to hatch normally. Egg masses deposited entirely within the pulp may be located externally a few days after oviposition

by a round white sunken area in the rind which varies in size with the passing of time up to an inch in diameter. Of 560 eggs found in the examination of the above-mentioned 85 Chinese oranges 471, or 84.1 per cent, were unhatched and dead. This same kind of mortality occurs to a less extent in ordinary Hawaiian limes, but with no regularity, as the rind of these fruits in most instances is so thick that the female can not place her eggs within the pulp.

MORTALITY AMONG LARVÆ

It has been shown that mortality among the eggs occurs in the rind and in the pulp. Larval mortality occurs chiefly during the first instar, either in the egg cavity or in the rag beneath. Though mortality does occur in the pulp to a slight degree, no further notice of it will be taken, as it has little bearing upon the general purpose of this paper. The data in Table I show that of 6,571 larvæ recorded as dead but 18 died in the pulp, while 3,166 died in the egg cavities where they hatched, and 3,387 in the rag of the rind. The causes for this mortality of larvæ in the rind are threefold: The oil from the ruptured cells, the texture of the walls of the puncture, and the texture of the rag.

In the treatment of the eggs with oil, as recorded in Table V, it was found that of the larvæ hatching from the 163 eggs out of the lot of 1,600 eggs sprayed all died either before they were entirely out of the eggshell or before they had crawled much more than one-fourth of an inch. They exhibited a general weakness entirely lacking in normal larvæ. Larvæ hatching from check eggs were normal and crawled actively to all parts of the containing vials. As the oil sprayed on the eggs and foliage on which the eggs rested appeared to have entirely evaporated by the time of hatching, the writers believe that the few larvæ that succeeded in emerging from the eggs died from weakness imparted to the developing embryo by the oil with which the eggs were sprayed rather than from the effect of any oil still on the foliage with which they came in contact on hatching. Subsequent experiments have shown this supposition to have been correct. The writers believe, therefore, that the very large percentage of the deaths among newly hatched larvæ occurring in the egg cavity is the result of the action of the oil liberated during the formation of the cavity—oil which is sufficiently abundant to weaken the developing embryo but not abundant enough to kill the egg.

To such weakened larvæ and probably to many other normal larvæ hatching in egg cavities made without the rupture of oil cells the texture of the walls of the cavity present another difficulty. In many host fruits, such as the peach and loquat, the eggs are crowded into and completely fill the cavity made by the female, but shortly after oviposition, possibly as the result of the action of a fluid introduced by the female with the eggs, the flesh of the host shrinks considerably from the eggs, thus usually

leaving the eggs well separated and with ample room, making conditions favorable for the newly hatched larvæ. (See figs. 1 and 2.) On the other hand, in citrous fruits no such enlargement of the egg cavity takes place. Instead there occurs a general hardening of the walls of the cavity, and the eggs remain as tightly packed as when deposited. In many instances the cavity walls become almost woody. In fact the egg cavities in all the thicker skinned citrous fruits, such as grapefruit, lemons, and sweet oranges, resemble a gall the cavity of which is filled with eggs and the opening more or less clogged with a yellowish substance from the ruptured cells, and in Hawaii still further sealed by exudations of gummy secretions, especially in certain grapefruit, limes, and lemons. These gall-like cavities in the rind do not share in the general withering of the rind that takes place in citrous fruits after they have been picked for some days, but stand out from the general surface as small nodosities. (See fig. 3.) It is usual in host fruits of the fruit fly for the punctured surface to develop a depression. These thickened and often woody walls of the cavity no doubt offer an obstacle to the larvæ reaching the rest of the fruit which they can not overcome; hence, the larvæ are forced either to die in the cavity itself or to work their way out through the opening of the puncture to the surface of the fruit. It is probably seldom that larvæ leave the fruit by way of the opening of the puncture, but a few newly hatched larvæ have been found by the writers with their bodies half way out of the fruit.

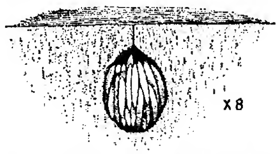


FIG. 1.—Cross section of peach, showing egg cavity of the Mediterranean fruit fly with eggs. Drawing made directly after oviposition. Original.

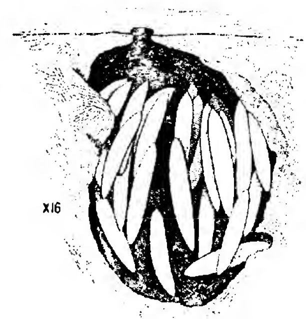


FIG. 2.—Cross section of peach, showing the general shriveling of the walls of the egg cavity and the separation of the eggs. Drawing made 1½ days after oviposition. Original.

Larvæ that succeed in getting out of the cavity must burrow through the rag before reaching the pulp, and this is a difficult task, as evidenced by the fact that out of 3,345 newly hatched larvæ that succeeded in reaching the rag, as shown in Table I,[1] 3,276, or 97.9 per cent, died in the rag. The larvæ, after leaving the egg cavity, burrow in all directions, but seldom get more than 1 inch from the cavity and usually not that far. Often they are able to reach the skin covering the pulp or to burrow

[1] This number excludes shaddock No. 1 and sour and Chinese oranges, which are of no commercial value and are more easily infested than grapefruit, lemons, limes, and sweet oranges.

down between the sections of the fruit, but seem to be lacking in strength to penetrate the skin after they have reached it. There apparently is nothing in the rag itself as a food to cause the death of the larvæ, as larvæ can attain full growth when feeding on the rag of certain shaddocks. Whether larvæ die or not seems dependent upon the degree of toughness of the rag, and the closeness with which the rag adheres to the skin covering the pulp. The toughest rag found was that of sweet oranges still hanging on the tree in March in a much overripe condition. These fruits had begun to be pithy at the stem end, and the rind, which was more or less russeted, had begun to wither and yielded no oil when sharply bent. These fruits were very much like the overripe, badly russeted seedling oranges frequently found on trees in Florida during April and May as "leftovers" from the winter crop. In the 20 fruits examined, containing an average of 7 punctures to the fruit, no larva was able to penetrate the rag. The coarsest rag or that with the loosest texture is that found in certain large shaddocks growing in Hilo, Hawaii (Pl. XLI). These fruits were much overripe when gathered from the tree in March. An examination of 14 of these fruits showed that out of 245 larvæ, mostly in the third instar, present in the rag and pulp, 152 were alive in the rag, 55 alive in the pulp, and only 38 dead, all in the rag. Fourteen other shaddocks, apparently in the same state of ripeness but growing on another tree and so very much undersized as to resemble a medium-sized grapefruit, had a very much tougher rag. An examination of these fruits showed that but 5 very young larvæ out of 701 found in the rag were alive and that no larvæ had succeeded in penetrating the pulp. The data in Table I show that in the grapefruit, lemons, limes, and sweet oranges examined, 326, 15, 424, and 1,552 first-instar larvæ, respectively, died in their attempt to puncture the rag, as compared with but 1 first-instar larva found alive in the rag of grapefruit and 17 third-instar larvæ found in the pulp of sweet oranges.

Fig. 3.—Section of grapefruit rind, showing two egg cavities, one in cross section. Drawing made one week after fruit was picked. Note conical elevation about the egg cavities left by the withering of the rind; also the thickened walls of the egg cavity and the single larval channel in the rag. Original.

The ordinary sour orange of Hawaii, which is identical with that grown in Florida, possesses a loosely attached rind the rag of which is much looser in texture and from the standpoint of imperviousness to the young larvæ seems half way between that of the ordinary sweet orange and large well-ripened shaddocks. After the larvæ have succeeded in passing through the rag of these oranges they work their way between the rag and the skin and finally enter the pulp, usually at the blossom end.

Hawaiian limes possess less rag than sour oranges and the larvæ reach the pulp more easily. In 1,692 ripe and yellow limes picked during April, showing an average of about 5 punctures to the fruit, the larvæ succeeded in reaching the pulp in but 287 cases. Chinese oranges have been shown to be generally infested because they possess a very thin, loosely fitting rind, and for practical purposes may be said to possess no rag. Unfortunately the writers have had but little experience with tangerines (*Citrus nobilis*), as these are rarely found in Hawaii, but such few fruits as have come to their attention have been well infested, which is to be expected, because of their thin, loosely fitting rind and rag.

PERSISTENT ATTACK LEADING TO INFESTATION OF THE PULP

Laboratory experiments and field examinations have shown that the female fly seldom deposits more than six eggs in a puncture at one time. So well has nature equipped the average citrous fruit to withstand attack that it is doubtful whether such fruits as the grapefruit, lemon, or orange would ever become infested [1] until very much overripe, if the female fly formed a new puncture for each batch of eggs deposited, thus making it necessary for the larvæ hatching from each lot of eggs to face identical difficulties in reaching the pulp. This, however, she does not always do. As many as 153 eggs have been taken from a single puncture in grapefruit. A very large number of punctures contained more eggs than the female deposits normally at one oviposition. It is very evident, therefore, that females oviposit in a large number of instances in the same puncture rather than make a fresh puncture for each batch of eggs. Frequently freshly laid eggs have been found in egg cavities from which channels made by larvæ from previously deposited batches of eggs extend through, to, and into the pulp or in punctured areas of the rind showing dry decay which is known from observation to have been forming for fully one month. Usually the rag beneath a puncture develops a discolored area, no matter whether the puncture originally contained eggs or not, and very often this discoloration of the rag, which appears to be caused by a dry rot, extends to the outer rind and causes deadened, sunken areas to form about the punctures. Such blackened areas, which had been developing in the rind of well-punctured oranges held at the laboratory for one month after picking, are shown in Plate XLII, figure 2.

It has already been stated that many larvæ die in the rag and that before dying some of these larvæ channel through the rag in all directions. Often all the larvæ escaping from a puncture will be found dead next the skin protecting the pulp; again they will be found dead at the heads of channels extending fully 1 inch from the puncture. In large shaddocks they may even channel 3 or 4 inches through the loose rag (Pl. XLI).

[1] The term "infested" is here applied to fruits which have larvæ in the pulp, show decay, and become generally unfit for consumption.

It is evident, therefore, that larvæ hatching from the successive batches of eggs deposited in punctures or in the decayed areas of the rind forming about the punctures find conditions increasingly favorable to their ultimate success in reaching the pulp. The longer the fruit is allowed to remain on the tree after it becomes ripe, the easier it is for the maggots to reach the pulp. The rind can not withstand indefinitely the persistent attack of successive lots of larvæ and the work of decay fungi to which the punctures give entry (Pl. XLII, fig. 1). Thus, 39 sweet oranges showing an average of 32 punctures to the fruit, gathered from the trees in September, 1913, at a time when they were just becoming ripe, developed no larvæ. On the other hand, out of 784 sweet oranges gathered during March, 1914, in a very much overripe condition, 254 produced 2,272 larvæ, or an average of about 9 larvæ to the fruit. On account of the looseness of the rind and rag of sour oranges and the greater ease with which the rind is destroyed by decay fungi, these fruits are more quickly infested by the fruit-fly larvæ.

While both sweet and sour oranges in an overripe condition ultimately succumb to the repeated attacks of the Mediterranean fruit fly if permitted to remain on the tree, lemons, both of the commercial smooth-skinned and the rough-skinned varieties, withstand these attacks with a constancy that is astonishing. Lemons are not grown in sufficiently large numbers in Hawaii to permit the writers to record observations on large quantities of fruit, but even in orchards where the fruit is heavily punctured infested fruits are very seldom found. In about two years' time only three infested lemons of the commercial variety and one of the rough-skinned variety have been seen by the writers or by fruit-fly inspectors. Out of 235 well-grown and for the most part ripe lemons of the commercial type, picked from the tree, only 1 developed larvæ (this contained 3), and this fruit when picked was partially decayed as a result of a thorn prick. Out of 161 lemons of the same variety, taken from the ground in a very much overripe condition, but 2 developed larvæ—1 and 5, respectively. No larvæ developed in 434 ripe rough-skinned but badly punctured lemons picked from the tree. One partially decayed rough-skinned lemon taken from the ground produced 12 larvæ.

The thicker skinned grapefruit, such as the writers have had an opportunity to study best, have shown a strong resistance to the repeated attack of larvæ or fungi. Yet these fruits were all grown on less than a dozen trees and in one garden. Twenty-five fruits taken from beneath these trees in a very ripe condition and showing an infestation of the rind equaling that recorded in Table I of the 39 fruits picked from the same trees, produced no larvæ in the pulp. However, larvæ have been found in a few thin-skinned grapefruit that were in a very much overripe condition.

SECONDARY ATTACK OF CITROUS FRUITS BY INSECTS OTHER THAN THE FRUIT FLY AND BY FUNGI

The excellent experimental work of the Bureau of Plant Industry carried on in Florida during the last few years has forcibly demonstrated the causes of decay of citrous fruits in transit from orchard to market. Mechanical injuries to the rind have been found to be a fertile source of trouble by furnishing entry for decay fungi. The writers believe that a great share of the decay of oranges en route to market, recorded in the early history of the Mediterranean fruit fly, was caused more by insanitary conditions in the holds of ships than directly by fruit-fly larvæ. It is more than likely that the oranges shipped from the Madeira Islands and the Azores to London contained fruit-fly punctures which greatly aided the blue mold in its destructive work.

Statements made by the early writers and even repeated in the Hawaiian Islands at the present time, that citrous fruits drop as soon as punctured, are untrue. There is no such thing as a general shedding of fruits following puncturing of the rind. Oranges and grapefruit have been known by the writers to hang on the tree from two to three months after they were first punctured. It is probable that the wholesale shedding of fruit recorded by others was caused by fungi or physiological troubles.

Species of Drosophila and Bruchus may usually be found ovipositing in breaks in the rind of Citrus made by the Mediterranean fruit fly. Their persistent attack, supplemented by decay fungi, causes an appreciable amount of decay in Hawaii.

EFFECT OF ATTACK OF THE MEDITERRANEAN FRUIT FLY UPON CITROUS CROPS OF CALIFORNIA AND FLORIDA

In the opening paragraph the writers made the statement that their investigations in Hawaii have led them to believe that even if the Mediterranean fruit fly should be introduced into the citrous regions of the United States it would not become a serious pest to citrous fruits. In the Hawaiian Islands, especially in the lowlands, climatic conditions are more favorable for the rapid increase of the fruit fly than they are in any section of the United States or of the Mediterranean regions where oranges are grown commercially. The monthly mean temperatures at Honolulu during 1912 and 1913 ranged from 69.6° to 79.2° F. During the hottest summer weather the fruit fly requires a minimum of about 14½ days to complete its life cycle from egg to adult. During late December, 1913, and January and early February, 1914, it required many flies fully 47 days to reach maturity in common guavas (*Psidium guajava*). During March and April, 1914, the fruit fly required from 20 to 30 days to pass from egg to adult in half-ripe peaches and from 28 to 40 days in lemons. In Bermuda during December and January,

when the monthly mean temperature normally ranges from 62.5° to 64.8° F., the senior writer, with the kind assistance of Mr. E. J. Wortley, Director of Agriculture, Bermuda Agricultural Station, found that the length of the pupal stage was about 31 days, which would make the period for development from egg to adult about 58 days in favored hosts. In Honolulu the cold-storage experiments of the writers have shown that the fruit fly requires about 91 days to complete the same development at about 56° F. A temperature of 54° to 57° will not prevent adults from emerging from pupæ in cold storage, although it lengthens the pupal stage from 8 days, a normal minimum required at Honolulu in warm weather, to 36 days. Very few eggs out of several hundred were able to hatch at a temperature of about 53° to 54°, while practically no eggs will hatch nor larvæ mature at a temperature of 50° F. A continued temperature ranging from 33° to 46° F. will kill pupæ and larvæ, although both may be subjected to these temperatures for short periods without apparent injury. Freezing temperatures have proved generally fatal to both larvæ and pupæ. At 45° larvæ are not able to pupate, although some hardy specimens may become active and pupate if removed at the end of a month from this temperature to the normal Honolulu summer temperature. A total of 10,203 second and third instar larvæ kept at a temperature varying from 42° to 46° were all dead at the end of 45 days, except one third-instar larva which was probably moribund, while out of 10,959 second and third instar larvæ kept at a temperature varying from 33° to 38° none were alive after the seventeenth day.

These data from the notes on file are given here to show that even the cool winter climate of the lowlands of Hawaii has a decided effect in checking the increase of the fruit fly, that temperatures as low as 56° F. greatly lengthen the life cycle, and that a temperature of 50° to 52° practically prevents eggs from hatching. Unfortunately no data are at hand on the effect of the temperature varying above and below a mean temperature ranging from 50° to 53°. Certain deductions, however, can be made from known facts regarding the development of the fruit fly in the Mediterranean region, especially in southern Spain, France, Italy, and Sicily, that show that the fly does not multiply, or at least undergoes an extremely slow development, when the monthly mean temperatures range from 50° to 54°. During the spring and summer of 1913, Prof. H. J. Quayle, of the University of California, investigated the status of the fruit fly in the Mediterranean regions for the Bureau of Entomology,[1] and his observations bore out the contentions of the present Italian entomologists that the fruit fly is not a serious pest to Citrus in Spain and Italy. The fact that Prof. Quayle found no evidence

[1] Quayle, H. J. Citrus fruit insects in Mediterranean countries. U. S. Dept. Agr., Bul. 134, 33 p., 2 fig., 10 pl. 1914.

of fruit-fly infestation in oranges and lemons in Spain during March or in southern Italy and in Sicily during April, May, and early June is strong evidence that the fruit fly is prevented by the mean temperatures prevailing in these countries from becoming a pest during the winter and spring months. Loquats are a preferred host of the fruit fly, being badly attacked when flies are present, and the appearance of infested fruits is such that infestation is easily detected. Even during July at Valencia, Spain, Prof. Quayle found but a slight infestation of peaches and overripe oranges. In August, near Palermo, Italy, peaches were found badly infested, but lemons growing in the midst of peach trees were not infested. Reports indicate that in Spain and southern Italy the fruit fly may cause some damage to ripening oranges during September and October, although this is slight and of short duration. Citrous fruits, especially oranges, are not usually punctured by the female fruit fly until they are well grown and about to turn color, and the period of time is short after they reach this stage of ripeness until cool weather renders the fly sluggish. The season of the year, therefore, when the bulk of the citrous crops are best suited for fruit-fly attack coincides with the season of inactivity of the fly due to lower temperatures.

The mean monthly temperatures given in Table VI indicate that the Mediterranean fruit fly would find conditions in the citrous regions of Florida and California quite similar to those in Spain and Sicily. The Florida temperature, especially in the citrous regions of southern Florida, is decidedly above the winter means of those of California. However, even if the temperatures were higher than they are, the writers feel that the fruit fly would assume a minor position as a citrous pest. The cool winter weather would have the same retarding effect upon development in California and Florida that it has in the European countries.

TABLE VI.—*Monthly mean temperatures in citrous regions*

Locality.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
Seville, Spain	52.2	55.9	59.5	63.9	69.6	78.1	84.7	84.0	78.1	68.4	60.1	52.9
Malaga, Spain	53.6	55.4	57.6	61.5	65.7	71.4	76.8	77.2	72.5	66.0	60.3	55.4
Naples, Italy	46.8	48.4	51.4	56.8	63.7	70.3	75.6	75.0	69.8	63.1	54.7	48.7
Palermo, Sicily	50.5	52.1	54.7	56.8	64.0	70.7	76.3	76.6	73.4	67.3	59.4	53.4
San Francisco, Cal	50.0	52.0	54.0	55.0	57.0	59.0	59.0	59.0	61.0	60.0	56.0	51.0
Redlands, Cal	51.0	52.0	55.0	61.0	66.0	74.0	78.0	78.0	72.0	65.0	59.0	53.0
San Diego, Cal	54.0	55.0	56.0	60.0	62.0	65.0	68.0	70.0	66.0	64.0	59.0	50.0
Portersville, Cal	49.8	51.6	59.0	......	......	......	......	......	......	66.0	57.0	48.0
Jacksonville, Fla	55.0	58.0	63.0	68.0	75.0	80.0	82.0	82.0	78.0	71.0	62.0	56.0
Eustis, Fla	58.0	61.0	67.0	70.0	77.0	81.0	83.0	83.0	80.0	73.0	66.0	60.0
Miami, Fla	65.0	67.0	71.0	74.0	76.0	81.0	82.0	82.0	81.0	78.0	74.0	69.0
Myers, Fla	62.0	65.0	68.0	72.0	77.0	80.0	81.0	81.0	80.0	75.0	70.0	64.0
Honolulu, Hawaiian Islands	71.4	70.8	69.6	72.6	74.6	76.0	77.4	78.2	78.2	77.6	74.6	74.0
Prospect Hill, Bermuda	62.5	62.2	63.9	66.1	71.4	77.7	79.8	81.0	78.0	73.7	68.6	64.6

The general effect of retarded development of the fruit fly due to cold weather is to increase the mortality among all stages. Even pupæ are subject to an increasing rate of mortality the longer they are subjected to lower temperatures. Adults seem more able to withstand prolonged cold weather than any of the other stages. One individual was kept alive by daily feeding for 4½ months during a Hawaiian winter and spring. However, during the cooler months the adults are more sluggish and fall more easily a prey to adverse climatic conditions, such as heavy winds and rains, and to predaceous insects. Mr. George Compere reports having seen adults sunning themselves on orange trees in Spain after a night during which the temperature dropped to freezing, thus showing that adults can withstand temporarily any cold snap likely to occur in a citrous section. However, the fact that adults do not succeed in thriving during the winter temperatures of southern Spain and Italy and in Sicily seems to be well proved by the fact that it is only during the summer and early fall that the fruit fly becomes a serious pest in favored host fruits and in overripe citrous fruits. If this were not so, fruits would become badly infested much earlier in the season than they do. The number of adults surviving the winter must be very small. Even the mild winters of Hawaii at Honolulu have a very noticeable effect upon the numerical abundance of the adult flies, as shown by trap experiments extending over one full year.

In addition to this beneficial effect of lower winter temperatures, both California and Florida growers will receive further protection as a result of the conditions surrounding the growing of Citrus as a commercial proposition. In the Hawaiian Islands, especially about Honolulu, citrous fruits are subjected to the most severe attack imaginable under field conditions. They are attacked over long periods by an abundance of fruit flies that mature in many host fruits ripening at intervals throughout the year on all sides of isolated citrous trees. The number of wild fruits in which the fruit fly can breed in the citrous regions of California and Florida is, in comparison with Hawaii, so extremely small that the fly would find conditions unfavorable for rapid increase, even if weather conditions were more favorable. In many instances large acreages of Citrus occur where vegetation is normally decidedly stunted unless irrigation is practiced. With the excellent work of the horticultural inspectors in California a reduction of the noncitrous host fruits in and about citrous groves is a practical proposition. Even near-by orchards of drupe fruits are not the menace that they seem to many, inasmuch as their crops are unsuitable for fly attack except during short periods of the year. The very scarcity of vegetation that can not be destroyed which produces fruits subject to fruit-fly attack makes it possible to attach a far greater importance in California and Florida than in the Hawaiian Islands to the excessive mortality of the fly discussed in this paper. It has been shown

that it takes repeated attacks to infest grapefruit, lemons, and oranges in Hawaii and that the pulp of these fruits is infested usually only after the fruits are very ripe; in fact, not until they become much riper than commercially-grown oranges usually are allowed to become in either California or Florida, unless exception be made of such varieties as late Valencias. The relatively small number of adult fruit flies entering a block of citrous trees would find it very hard to establish themselves, since the numbers would be so insignificant as compared to the fruit surface suitable for oviposition that each female would be less likely to oviposit repeatedly in the same puncture. Her progeny would therefore meet with almost insurmountable difficulties in reaching the pulp.

It has been stated that in Hawaii citrous fruits offer themselves for attack over several months and that they are not subject to serious attack until they have turned or are about to turn color. It is a well-known fact among horticulturists that in very equable climates the pulp of oranges may be ripe enough to eat while the rind is still very green. In Florida and California this is not so true. One has only to visit the packing houses in either State to be convinced that much fruit is gathered for the early trade in a semiripe condition, or at least when the rind is quite green in color. The writers feel safe in saying that market conditions are such that early fruit is placed on the market at the earliest possible moment, in order that high prices may be secured. The fear of unseasonable frost and freezes has made it difficult for those who have the interests of the citrous industry at heart to prevent the shipping of too green fruit. It would seem that with a reasonable expenditure of more care than labor, citrous groves in either Florida or California can be made so well protected from the Mediterranean fruit-fly attack that such few flies as enter them during the fall will find the early fruit, upon which they can work because of its degree of ripeness, picked before they are able to injure it to any extent. The cold weather will protect the later fruit by rendering the fruit flies inactive, and by the time the spring temperatures become suitable for fly activity the bulk of the fruit will have been marketed and the numerical abundance of the adult flies greatly lessened.

In addition, if it becomes necessary, as a result of unfavorable conditions, to use artificial means of control, spraying with a cheap poisoned bait will be a practical method of reducing the number of adults. If the writers under most adverse conditions can reduce by spraying the number of adult fruit flies over 50 per cent in one city block in Honolulu, into which it has been proved that adults are continually migrating, it is only reasonable to expect that the same good results as have been secured in South Africa, where fruit has been protected by spraying, will follow spraying in either Florida or California, where outside sources of infestation can be so easily controlled.

CONCLUSION

Citrous fruits are not the favored host fruits of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) that the earlier writers thought. While grapefruit, oranges, lemons, and many limes may become quite badly infested with well-grown larvæ if allowed to remain on the tree long after they become sufficiently ripe for the market, nature has so well equipped them to withstand attack that larvæ are seldom found in their pulp until they are much overripe. Oranges and grapefruit are generally eaten and found uninfested if gathered as they ripen. Indeed, in Honolulu, where conditions are very favorable to early infestation of the pulp, owing to the excessive numbers of adult flies breeding in a large number of host fruits ripening in rapid succession, it is doubtful whether grapefruit, oranges, and lemons would ever become infested until long after becoming overripe if the female fly formed a fresh egg cavity for each batch of eggs deposited, for the reason that the eggs and the young larvæ found in the egg cavity and in the rag of the rind would then be forced always to face well-nigh insurmountable difficulties. The oil of the cells ruptured in the formation of the egg cavities kills a large percentage of the eggs and newly-hatched larvæ. Larvæ that succeed in entering the rag from the egg cavity are able to reach the pulp in astonishingly small numbers because of the imperviousness of the rag. It is only the persistent attack of successive lots of larvæ hatching from different batches of eggs laid in the same puncture in which the oil has become inoperative that finally breaks down the barrier between the young larvæ and the pulp.

The Mediterranean fruit fly is quickly affected by low temperatures. A temperature of about 56° F. has lengthened the time required by the fly to pass from the egg to the adult stage from 14½ to 91 days. A temperature ranging from 50° to 55° F. will either seriously check development or kill large numbers of the immature stages of the fly. The winter monthly mean temperatures of California and Florida are so similar to those of the citrous regions of southern Spain and Italy and of Sicily that it is to be expected that the fruit fly, if introduced to the mainland, would not become a serious pest to *Citrus* spp. It happens that the very cold temperature necessary to bring citrous crops to that degree of perfection in which they are most susceptible to fruit-fly attack likewise renders the fly so inactive or sluggish that it may be disregarded as a pest for that period of the year.

In addition to the assistance of adverse climatic conditions during that part of the year when they are most needed to protect citrous crops, the growers of California and Florida are still further protected—and most admirably so—from attack by the very scarcity of wild host fruits that can not be destroyed. It will be found a practicable undertaking to remove such a number of noncitrous host plants at present

growing about commercial citrous orchards that the succession of fruits in which the Mediterranean fruit fly can breed during the large portion of the year when citrous fruits are unavailable for attack because of their greenness will be reduced to a minimum, if not entirely done away with. It is under conditions such as can be secured in California and Florida that the excessive mortality occurring in the rind will become a valuable factor in preventing infestation or establishment of the pest, as each fruit will in reality become a trap for stray females. The scarcity of host fruits will also make spraying with poisoned baits a practical undertaking, should it become necessary to resort to artificial methods of control.

Adverse climatic conditions at a season when citrous fruits are most susceptible to attack, solid plantings of Citrus in commercial orchards, a scarcity of noncitrous host fruits, the ease with which the fly can be reduced by spraying with poisoned baits, and the general practices followed in harvesting fruits make it possible for the citrous growers of California and Florida to rest assured that the discovery of the Mediterranean fruit fly in either State will not bring about the ruination of the industry. Its presence will be a constant menace, but it can be successfully fought.

LITERATURE CITED

1817. LATREILLE, P. A. Les Tephrites. *In* Cuvier, G., La règne animal, v. 3, p. 647. Paris.

1829. MACLEAY, W. S. Notice of *Ceratitis citriperda*. an insect very destructive to orange. *In* Zool. Jour. [London], v. 4, p. 475-482, pl. 15, 1829.

1842. BRÊME, F. DE. Note sur le genre *Ceratitis* de M. MacLeay. *In* Ann. Soc. Ent. France, v. 11, p. 183-190, pl. 7, fig. 1-5, Séance du 4 mai, 1842.

1848. WESTWOOD, J. O. The orange fly. *In* Gard. Chron., 1848, no. 37, p. 604, 3 fig.

Account of *Ceratitis capitata* and character of injury.

1859. GOUREAU, COL. *In* Ann. Soc. Ent. France, sér. 3, v. 7, Bul., p. XLIII-XLV, Séance du 23 mars, 1859.

Communication on *Ceratitis hispanica* reared from an orange sent to M. Goureau by M. Villeneuve.

1870. RONDANI, C. Ortalidinae italicae collectae, distinctae et in ordinem dispositae . . . Dipterologiae italicae prodromi, Pars VII. fasc. 4. *In* Bul. Soc. Ent. Ital., v. 2, p. 29, 1870.

Description of *Petalophora* Macq.

1871. LABOULBÈNE, A. Note sur les dommages causés par la *Ceratitis hispanica* aux fruits des orangers dans nos possessions d'Algérie. *In* Ann. Soc. Ent. France, sér. 5, v. 1, p. 439-443, Séance du 13 décembre, 1871.

PLATE XI.

Fig. 1.—Orange infested with larvæ of the Mediterranean fruit fly (*Ceratitis capitata*). Note that the fruit looks sound, except about the irregular hole, through which a few well-grown larvæ have already left the fruit. Original.

Fig. 2.—Orange infested with larvæ of the Mediterranean fruit fly (*Ceratitis capitata*), showing two breathing holes of the larvæ in the decayed area. Original.


1
2

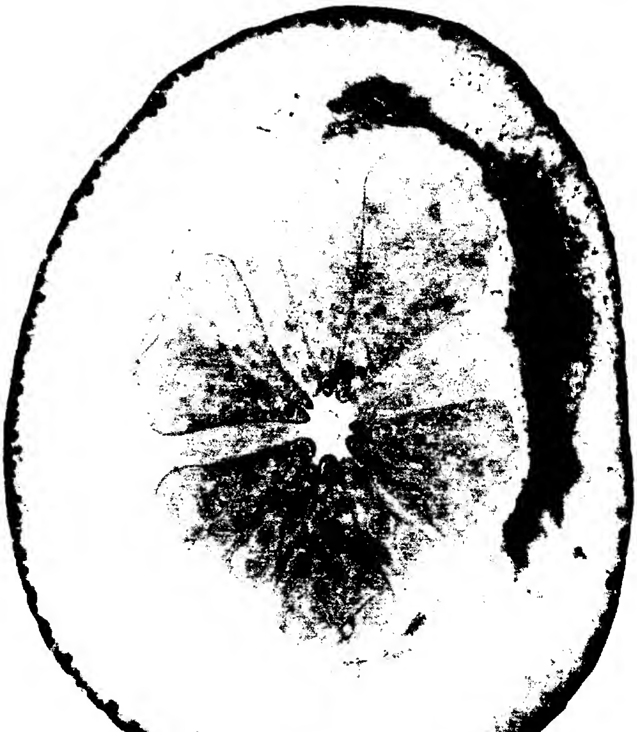


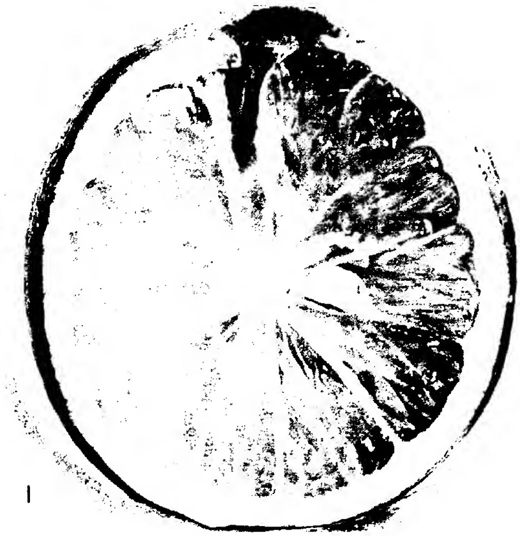
PLATE XLI

Cross section of shaddock No. 1, showing the thick, loose texture of the rag with darkened area above and to the right showing the channels made by well-grown Mediterranean fruit-fly larvæ. Original.

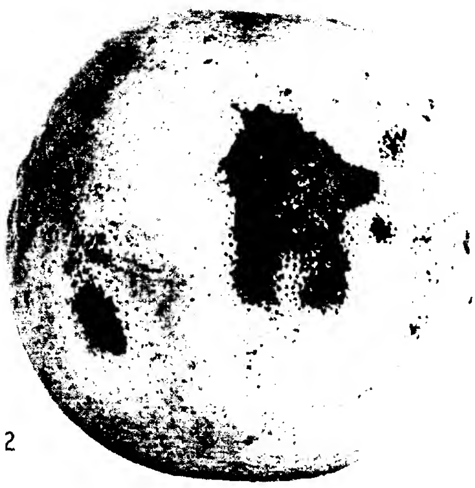
PLATE XLII

Fig. 1.—Cross section of the orange shown on Plate XL, figure 2. Note that in this instance the larvæ have brought about decay in only one section. Often many sections are thus affected in very ripe fruits. Original.

Fig. 2.—Orange containing 87 punctures in the rind. Photographed one month after being picked from the tree in a ripe condition. Note that the rind about many punctures is sunken as a result of a dry black-rot. The pulp of this fruit was perfectly sound. Original.



1



2

PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE

By Heinrich Hasselbring and Lon A. Hawkins, *Plant Physiologists, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry*

INTRODUCTION

In resting storage organs of plants growing in northern and in temperate regions, carbohydrate transformations involving the disappearance of reserve starch during the colder months and its temporary reappearance in spring have been found to be of general occurrence. The disappearance of starch from the cortex of trees in winter and its reappearance in early spring was first noted by Müller (1877),[1] who believed the absence of starch in the cortex resulted from its migration into the wood. Russow's investigations (1882–83), which included the examination of a number of tropical and subtropical greenhouse plants, showed that the total or partial disappearance of starch from the cortex of woody plants in winter was a phenomenon of widespread occurrence. He found, however, that the starch did not migrate into the wood, as Müller supposed, for when pieces of cortex chiseled from trunks of trees were kept at a temperature of 14° to 17° R. (17° to 21° C.) starch grains began to reappear in 20 hours. In the tissues which were free from starch in winter he found oil and fats. He observed a correlation between the temperature and the disappearance and reappearance of starch, but since the processes occurred also in tropical plants in the greenhouse, he did not regard temperature changes or climatic conditions as the prime causes of the observed transformations. Later Grebnitzky (1884) and Baranetzky (1884) showed that the starch of soft-wooded trees disappeared entirely from the wood, cortex, and rays in winter, and that oil appeared in its place, while in hardwood trees the starch disappeared from the cortex, but persisted in the wood. Fischer (1891), in his extended investigations on the physiology of woody plants, fully confirmed the observations of Russow (1882–83), Grebnitzky (1884), and Baranetzky (1884) regarding the appearance of oil in place of starch in soft-wooded trees, and showed further that in hardwood trees glucose and tannin are present in the cortex after the disappearance of the starch, and that the glucose, but not the tannin, disappears when starch is regenerated. He found that the regeneration of starch takes place at a temperature only a few degrees above 0° C. The minimum temperature at which he observed the regeneration of starch in twigs was about 5° C., while at 10° to 20° the process went on very rapidly.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 341.

That the periodic transformation of reserve starch is not restricted to the stem tissues of plants is shown by the observations of Haberlandt (1876), of Mer (1876), and of Schulz (1888), who found that the starch disappears from evergreen leaves in temperate regions in winter, while Haberlandt and Schulz noted also that it was re-formed in spring. The most thorough investigation of the carbohydrate transformations in evergreen leaves was made by Lidforss (1907), who found that the leaves of all evergreen plants in cold countries, except aquatic plants, lose their starch in winter, sugar appearing in its place, and that starch is regenerated in the leaves in February and March when the temperature scarcely rises above 5° C.

That similar changes occur in the subterranean parts of perennial plants in temperate regions was shown by Rosenberg (1896), who observed the disappearance of starch after leaf fall in the subterranean parts of *Spiraea ulmaria*, *Scrophularia nodosa*, *Plantago major*, *Potentilla argentea*, and *Hepatica triloba*, but did not determine what substances appeared in its place. By far the most complete account of carbohydrate transformations in dormant organs of this type is given by Müller-Thurgau (1882) in his classical researches on the accumulation of sugar in the potato (*Solanum tuberosum*) and other plant organs at low temperatures. Müller-Thurgau found that an accumulation of sugar and a corresponding loss of starch occurred in potatoes kept at low temperatures (0° to 6° C.), while, contrary to popular opinion, no sugar is formed in potatoes which have been actually frozen. He found that when potatoes which had become sweet as a result of exposure to low temperature are kept at a higher temperature (8° to 10° C.) the sugar disappears and the starch increases. Furthermore, he showed that the sugar formed consists mostly of reducing sugar with some cane sugar in the proportion of about 2.5 to 1, and that similar transformations occur in other parts of plants. These phenomena are interpreted by Müller-Thurgau (1882) as follows:

The transformation of starch into sugar is an enzymic process which, although more rapid at high temperatures, occurs also at low temperatures. The respiratory activity which is almost at a standstill at 0° C. rises with the temperature so that at higher temperatures an increasingly greater amount of sugar is consumed by respiration. The amount of sugar used in respiration at higher temperatures is, however, small compared with that utilized by another process—i. e., the re-formation of starch from sugar, which takes place at temperatures somewhat above 0° C. and increases in speed with the rise of temperature. Appleman (1914) in his studies on the rest period of the potato also finds that the carbohydrate changes in the dormant tubers are entirely dependent upon changes of temperature. It appears, therefore, that the carbohydrate transformations of the potato, although a

subtemperate plant and not capable of long withstanding temperatures much below freezing, resemble in their general trend those of subterranean organs of temperate plants. In the more strictly tropical sweet potato (*Ipomoea batatas*) carbohydrate transformations of a similar nature have been observed. Thus, Harrington (1895) found that in stored sweet potatoes there was an increase of the total amount of sugar up to March 6, beyond which the experiments were not continued. Shiver (1901), whose experiments were somewhat more extensive, found that during the time of his experiments (up to April 17) there was a gradual decrease of starch and an increase of cane sugar, while the invert sugar showed but slight fluctuations. Neither of these writers described the conditions under which the potatoes were stored nor attempted to determine the effect of temperature on the metabolic changes.

The storage of sweet potatoes is accompanied by considerable losses as a result of decay which is not wholly preventable by any of the methods of storage advocated at present. The decay is brought about by microorganisms which invade the tissues. In the matter of susceptibility the internal changes in the roots must play an important part. These changes are affected by changes in temperature and other conditions to which the roots are subjected during storage. It is therefore a matter of practical importance, as well as of theoretical interest, to study the internal changes which take place in sweet-potato roots after harvest and during storage, and to determine the effect of external conditions upon such changes. The work reported in this paper is a general study of the carbohydrate metabolism of sweet potatoes stored at different temperatures.

PLAN OF THE EXPERIMENTS

For the purpose of this work two varieties of sweet potatoes, the Jersey Big Stem, representing the sugary type, and the Southern Queen, representing the starchy type, were selected. The potatoes used in the experiments were a part of the general crop grown by the Office of Horticultural and Pomological Investigations during the summer of 1911 in a series of variety tests which had been continued for a number of years. At the time of harvesting, a representative lot of about 15 bushels of each of the two varieties was selected in the field and packed in slat crates holding about a bushel each. These were placed with the rest of the crop in the sweet-potato cellar of the Office of Horticultural and Pomological Investigations, where all were subjected to the "sweating," or curing, process. During the period of curing, the temperature of the room was kept at approximately 27° C. for about 10 days, after which it was allowed to drop to the regular storage temperature, ranging in this case, except near the end of the season, between 11.7° and 16.7° C. Nine crates of each variety were left in the cellar at the above-mentioned

temperature, which was maintained by the aid of artificial heat when necessary. The remaining six crates of each variety were placed in a cold-storage room, which was kept at a fairly uniform temperature of 4° C., by means of circulating brine cooled by ice and salt. Thus, both the lot stored at the usual storage temperature and that placed in cold storage were submitted to the same preparatory curing process. In order to determine the carbohydrate changes which occurred in these lots during the season, samples were analyzed on the day the potatoes were dug and at intervals of about a month during the course of the experiment, from October to June. In these samples the water, starch, reducing sugar, and total sugar were determined.

EXPERIMENTAL METHODS

SAMPLING.—For each set of determinations, a random sample of 4 to 5 kg. was taken. The roots were rapidly washed and wiped with a towel. When the surface had become entirely dry the roots were cut up as quickly as possible and ground in a power-driven meat grinder having a face plate with holes 3.2 mm. in diameter. The operation of cutting and grinding required about 10 minutes. The mash thus obtained was thoroughly mixed on a glass plate and quartered twice. The final sample thus obtained was placed in a crystallizing dish and covered with a damp towel while the samples for sugar, starch, and moisture determinations were being weighed out.

MOISTURE.—For the determination of moisture, samples of approximately 10 gm. were transferred into tared weighing bottles and accurately weighed. The material was covered with 95 per cent alcohol, which was subsequently evaporated in vacuum desiccators containing sulphuric acid. The samples were then dried to their lowest weight in a current of hydrogen in a vacuum oven at 78° C. The drying required 15 to 18 hours, during which the bottles were weighed three or four times.

STARCH.—It was not possible to make the starch determinations immediately. Samples of 25 gm. correctly weighed to 1 cm. were therefore transferred to Erlenmeyer flasks of 200 or 250 c. c. capacity and covered with 150 c. c. of 95 per cent alcohol. A little precipitated calcium carbonate was added to the flasks, which were then brought to the boiling point in a water bath. Subsequently the samples were washed with alcohol into tared porcelain extraction thimbles, 75 mm. high and 40 mm. in diameter, with perforated bottoms which were covered with filter paper cut to fit. Another piece of filter paper was pressed down upon the material and held in place by means of a cotton plug. The thimbles were supported well up in Soxhlet extraction apparatus and extracted with strong alcohol for 12 hours. After extraction the cotton and filter paper were removed, and the thimbles were

dried for 20 hours at 60° C. and subsequently were allowed to stand in the laboratory at least 48 hours, in order that the material might come to a state of moisture-equilibrium with the air. The thimbles were then weighed and the material was quantitatively transferred to a mortar and ground to a fine powder. The starch was determined as glucose by the acid-hydrolysis method (Wiley, H. W., et al., 1908) in two accurately weighed fractions of this powder, each representing about one-half of the extracted residue before it was ground.

SUGAR.—For the determinations of sugar, samples of 25 gm. were washed into 250 c. c. volumetric flasks with enough neutral 70 per cent alcohol to bring the volume up to about 200 c. c. About 1 gm. of calcium carbonate was added to each flask. The flasks were then boiled in the water bath for 10 minutes and on the following day were cooled to 20° C. and filled to the mark. After being stoppered they were allowed to stand for a few days, during which they were occasionally shaken to insure uniformity of concentration of sugars in the solid and the liquid portions of the contents. The solutions were subsequently treated essentially according to the method described by Bryan, Given, and Straughn (1911). Reducing sugars and total sugars were determined according to the method of Allihn (Wiley, H. W., et al., 1908). The cane sugar was calculated from the difference between the total sugar and the reducing sugars.

TEMPERATURE.—The temperature of the two storage rooms was recorded by thermographs. The curves obtained in the warm storage room were integrated with a planimeter to obtain the average weekly temperatures, which are given in Tables I and II. The average weekly temperatures for the cold-storage room were written down from inspection of the records, since the tracings in this case were practically straight lines.

EXPERIMENTAL DATA

The data showing the seasonal changes in the composition of sweet potatoes stored in the farm cellar at a temperature varying mostly from 11.7° to 16.7° C. are given in Table I. The percentages of carbohydrates have all been referred to the original moisture content of the potatoes. The loss of solid matter by respiration had, of course, to be disregarded. The numbers expressing the total content of carbohydrates were obtained by the addition of the numbers representing the starch (as glucose) and the total sugars (as glucose).

TABLE I.—*Carbohydrate transformations in sweet potatoes stored in the farm cellar*

BIG STEM

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).[a]	Gain or loss of sugar (as glucose).[a]
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*
Oct. 20	73.50	19.07	1.90	0.90	2.90	24.09	....	....
Nov. 8	72.99	16.94	3.51	1.32	5.02	23.85	−2.36	+2.12
Dec. 6	71.89	16.42	3.91	1.40	5.55	23.79	− .58	+ .53
Jan. 4	72.06	16.02	4.39	1.28	5.90	23.70	− .44	+ .35
Feb. 1	72.18	14.11	6.06	1.67	8.04	23.71	−2.12	+2.14
Mar. 1	71.97	13.09	6.96	1.44	8.76	23.31	−1.13	+ .72
Mar. 20	73.02	13.44	6.40	1.10	7.84	22.77	+ .39	− .92
Mar. 26	72.49	14.47	5.61	.87	6.77	22.85	+1.14	−1.07
Apr. 16	72.87	14.20	6.03	.90	7.24	23.02	− .30	+ .47
June 1	72.45	14.62	5.85	.87	7.02	23.77	+ .47	− .22

Average weekly temperature (Big Stem):

°C.	Week ending—
20.7	Oct. 30
21.7	Nov. 6
16.7	Nov. 13
16.7	Nov. 20
15.6	Nov. 27
15.0	Dec. 4
15.0	Dec. 11
18.3	Dec. 18
15.6	Dec. 25
14.4	Jan. 1
12.8	Jan. 8
11.7	Jan. 15
12.8	Jan. 22
16.1	Jan. 28
15.0	Feb. 5
11.7	Feb. 12
[b] 12.2	Feb. 19
16.7	Feb. 26
14.4	Mar. 4
[b] 15.0	Mar. 11
15.0	Mar. 18
16.1	Mar. 25
16.1	Apr. 1
18.9	Apr. 8
16.7	Apr. 15
17.8	Apr. 22
20.6	Apr. 29
18.9	May 6
18.9	May 13
17.2	May 20
21.1	May 27
21.1	June 3

SOUTHERN QUEEN

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).[a]	Gain or loss of sugar (as glucose).[a]
Oct. 23	71.69	22.09	1.19	0.39	1.64	26.18	....	....
Nov. 10	68.41	19.87	2.97	.77	3.89	25.96	−2.47	+2.25
Dec. 7	67.69	19.30	3.50	.72	4.41	25.85	− .61	+ .52
Jan. 11	67.51	19.75	3.53	.75	4.46	26.41	+ .50	+ .05
Feb. 3	68.02	19.22	3.95	.60	4.75	26.11	− .59	+ .29
Feb. 28	68.00	18.99	4.05	.53	4.80	25.90	− .26	+ .05
Apr. 8	66.71	20.35	2.93	.52	3.61	26.22	+1.51	−1.19
May 4	69.21	19.78	3.39	.51	4.07	26.05	− .63	+ .46
June 4	68.15	20.15	2.80	.55	3.50	25.89	+ .41	− .57

Average weekly temperature (Southern Queen):

°C.	Week ending—
20.7	Oct. 30
21.7	Nov. 6
16.7	Nov. 13
16.7	Nov. 20
15.6	Nov. 27
15.0	Dec. 4
15.0	Dec. 11
18.3	Dec. 18
15.6	Dec. 25
14.4	Jan. 1
12.8	Jan. 8
11.7	Jan. 15
12.8	Jan. 22
16.1	Jan. 28
15.0	Feb. 5
11.7	Feb. 12
[b] 12.2	Feb. 19
16.7	Feb. 26
14.4	Mar. 4
[b] 15.0	Mar. 11
15.0	Mar. 18
16.1	Mar. 25
16.1	Apr. 1
18.9	Apr. 8
16.7	Apr. 15
17.8	Apr. 22
20.6	Apr. 29
18.9	May 6
18.9	May 13
17.2	May 20
21.1	May 27
21.1	June 3

[a] Per 100 gm. of material.

[b] Record obtained for only one day of this week.

The data in Table I show that under the conditions of this experiment the moisture content of the roots remains fairly constant. There is a slight decrease in the moisture content, more marked in the Southern Queen than in the Big Stem variety, during the curing process, but on the whole there is comparatively little change in the percentage of moisture. The loss of moisture is probably compensated in part by the water formed by respiration, while the loss of substance by respiration would increase the relative moisture content, thus tending to conceal actual water lost.

The percentage of starch shows a rather sudden decrease immediately after the potatoes are dug. The subsequent decrease is more gradual, and continues until a minimum is reached in March. After that time there is a continuous rise in the percentage of starch until the last date on which the potatoes were examined.

Concomitant with the changes in the percentage of starch there is an inverse change in the percentage of sugar. Corresponding with the first sudden decrease of starch, there is an equally sudden increase in sugar. Later the increase in sugar content is more gradual, and reaches a maximum at the time of the starch minimum. After the sugar content has reached a maximum there is a gradual decrease, which, however, is not as marked as the increase during the first part of the season.

The course of the changes in the percentage of cane sugar follows that of the total sugar in both varieties, but in the Southern Queen the invert sugar after the initial rise shows an almost continuous decrease, whereas in the Big Stem the invert sugar content also shows a distinct maximum.

The total carbohydrate content in both types remains fairly constant; consequently the numbers showing the loss (or gain) of starch between the successive dates of sampling show a fairly close agreement with those showing the corresponding gain (or loss) of total sugar. The aberrations are probably to be attributed partly to the loss of substance through respiration, but mostly to nonconformity of samples.

The data showing the carbohydrate transformation in sweet potatoes stored at low temperatures (approximately 4° C.) are given in Table II.

TABLE II. *Carbohydrate transformations in sweet potatoes in cold storage*

BIG STEM, FIRST LOT[a]

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).[b]	Gain or loss of sugar (as glucose).[b]	Average weekly temperature.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*	°*C.*	*Week ending—*
									7.8	Oct. 23
Nov. 8	72.99	16.94	3.51	1.32	5.02	23.85	....	....	7.2	Oct. 30
									5.6	Nov. 6
									5.6	Nov. 13
Dec. 6	72.99	13.31	6.46	2.02	8.82	23.02	−4.03	+3.80	4.4	Nov. 20
									4.4	Nov. 27
									4.4	Dec. 4
									3.9	Dec. 11
Dec. 21	70.77	10.80	7.33	1.60	9.31	21.31	−2.70	+ .49	3.3	Dec. 18
									2.8	Dec. 25

[a] The figures are all calculated for the original water content of the roots, 73.50 per cent.
[b] Per 100 gm. of material.

TABLE II.—*Carbohydrate transformations in sweet potatoes in cold storage*—Continued

Date.	Water.	Starch.	Cane sugar.	Reducing sugar as glucose.	Total sugar as glucose.	Total carbohydrates as glucose.	Gain or loss of starch (as glucose).	Gain or loss of sugar (as glucose).	Average weekly temperature.	
									°C.	Week ending—
BIG STEM, SECOND LOT[a]										
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*Gm.*		
Mar. 27	72.19	12.99	6.41	1.65	8.39	22.82	....	....	....	....
Apr. 30	73.32	9.74	8.74	2.44	11.64	22.47	−3.61	+3.25	....	....
SOUTHERN QUEEN[b]										
									7.8	Oct. 25
Nov. 10	68.41	19.87	2.97	0.77	3.89	25.96	....	....	7.2	Oct. 30
									5.6	Nov. 6
									5.6	Nov. 13
									4.4	Nov. 20
Dec. 8	66.77	17.40	5.93	.59	6.83	26.16	−2.74	+2.94	4.4	Nov. 27
									4.4	Dec. 4
									3.9	Dec. 11
Dec. 22	67.57	16.48	6.94	.65	7.96	26.28	−1.02	+1.13	3.3	Dec. 18
									2.8	Dec. 25

[a] The figures are all calculated for the original water content of the roots, 73.50 per cent.
[b] The figures are all calculated for the original water content of the roots, 71.69 per cent.

In these experiments three lots of potatoes were used. One lot of the Big Stem and one of the Southern Queen were placed in cold storage immediately after they had been cured. Another lot of the Big Stem variety which had been kept in warm storage until March 27 was placed in cold storage on that date. The cold-storage experiments were of short duration, since the potatoes invariably rotted after having been kept at the low temperature for about six weeks.

These data show that at low temperatures the disappearance of starch and the accumulation of sugar in sweet potatoes take place more rapidly and proceed to a greater extent than at high temperatures. As to the relative proportion of the individual sugars, the two types of potatoes seem to differ somewhat. In both types the cane-sugar content is markedly higher in cold than in warm storage. In the Big Stem sweet potatoes the invert-sugar content also is higher in cold storage, but in the Southern Queen the invert-sugar content is no higher in cold than in warm storage. In general, cane sugar is the chief product which accumulates at low temperatures. The total carbohydrate content, with one exception, remains fairly constant, and the increase of sugar accounts for the loss of starch. The exception mentioned is the discrepancy between the loss of starch and the gain of sugar in the Big Stem potatoes during the interval from December 9 to December 21. The only explanations that can at present be suggested for this discrepancy are either that after long exposure to low temperatures the various phases in the process of the transformation of starch into sugar are influenced in such a way that intermediate products which escape detec-

tion by the analytical methods employed accumulate to a greater extent than usual; or, inasmuch as many of the potatoes showed small rotten spots at the time of the last sampling, it is possible that although these were cut out and the flesh appeared otherwise entirely sound, the enzyms secreted by the fungus had brought about a partial transformation of starch beyond the zone actually invaded by the mycelium.

DISCUSSION OF RESULTS

A striking fact brought out in the tables is the high starch content and the low sugar content of the sweet potato immediately after harvesting. A number of analyses, not here reported, of potatoes dug at different times also showed that freshly dug potatoes contain only small quantities of sugar. However, as soon as the potatoes are dug, a rapid transformation of starch into sugar takes place. A number of experiments not given here showed that this sudden transformation of carbohydrates takes place over a wide range of temperatures and that even at 30° C. the process is so rapid that sugar accumulates in excess of the quantity used in respiration, while at any subsequent period the accumulated sugar diminishes at that temperature as a result of respiration. This initial transformation is in such striking contrast with the later less rapid transformation that the two may almost be considered as distinct phases in the carbohydrate metabolism of the roots. It appears that during the period of active growth processes occur which prevent the accumulation of sugar in the roots. The elaborated materials from the leaves are almost wholly transformed into starch. The reverse process, which takes place as soon as the potatoes are dug, seems to be associated with the cessation of the flow of materials from the vines to the roots. The influx of materials from the vines therefore seems to determine the direction of the carbohydrate transformation in the growing roots.

Subsequent to the initial period, the carbohydrate transformations in the sweet potato are greatly influenced by temperature. In warm storage there is a continual accumulation of sugar in excess of the quantity used for respiration during the first part of the storage period. The corresponding disappearance of starch leaves no doubt as to the source of the sugar.

During the latter part of the season the process is apparently reversed. The increase in the percentage of starch and the decrease in the percentage of sugar during this period suggests that during the latter half of the storage season a re-formation of starch takes place, such as has been observed in twigs and woody stems and in the tubers of the common potato.

It should be noted, however, that increased respiration during the latter half of the season, during which the temperature of the storage room rose gradually, may account for the loss of sugar. However, the constancy of the total carbohydrates and the increase in the percentage of starch seem

to show that there is an actual transformation of sugar to starch during this period. In a general way the course of the carbohydrate transformations in sweet potatoes seems to be correlated with the seasonal variation in the temperature of the storage room. That the temperature may be the controlling factor in determining the direction of the carbohydrate transformation is shown by the continuous transformation of starch into sugar in the sweet potato as well as in other storage organs of plants at low temperatures and the reversion of the process at higher temperatures. Further experimentation is necessary, however, in order to determine whether temperature is the sole controlling factor.

In some respects the behavior of the sweet potato is in marked contrast to the behavior of resting storage organs of plants of temperate regions. In general, it has been found that the accumulation of sugar as a result of starch transformation ceases at temperatures only a few degrees above 0° C. Thus, Fischer (1891) found that in the cortex of trees the regeneration of starch takes place at a temperature a few degrees above 0° C., while Müller-Thurgau (1882) found that in the common potato the accumulation of sugar practically ceases at 8° C. In the sweet potato a rapid transformation of starch into sugar in excess of the quantity used for respiration takes place in freshly dug potatoes at temperatures as high as 30° C. At later periods a marked accumulation of sugar takes place in the sweet potato at temperatures much higher than those at which the accumulation of sugar ordinarily ceases in resting storage organs.[1]

The sweet-potato roots exhibit a further peculiarity with respect to the quantitative relations of the substances formed by the conversion of starch. With the exception of soft-wooded trees, where oil results from the conversion of starch, reducing sugars have been observed as the most usual and most abundant products resulting from starch transformation in resting storage organs. In the common potato Müller-Thurgau (1877) found that cane sugar is present together with glucose in the proportion of 1 part of cane sugar to 2.5 parts of glucose, while Appleman (1914) reports in potatoes kept at a temperature around 0° C. for 2½ months 3.94 per cent of total sugar and 2.40 per cent of reducing sugar. In the sweet potato cane sugar is the principal product formed by the conversion of starch, while the quantity of reducing sugar is small. In warm storage the cane-sugar content of the Big Stem sweet potatoes reached 6.96 per cent and that of the Southern Queen 4.05 per cent, while the maximum reducing sugar contents were, respectively, 1.67 and 0.77 per cent. In cold storage the cane-sugar content of the two types rose to 8.74 and 6.94 per cent, respectively, while the maximum reducing sugar content in the two cases was 2.44

[1] The transitory solution initiating the process of translocation of starch in leaves and in the storage organs of plants about to resume active growth, of course, takes place at higher temperatures. This process seems to be somewhat different in its nature from the solution of starch in resting storage organs as a result of exposure to low temperatures.

and 0.77 per cent, the invert sugar content of the Southern Queen having shown no increase in cold storage. In all cases the proportion was approximately 4 to 5 parts of cane sugar to 1 of reducing sugar.

SUMMARY

During its growth the sweet-potato root is characterized by a very low sugar content. The reserve materials from the vines are almost wholly deposited as starch.

Immediately after the roots are harvested there occurs a rapid transformation of starch into cane sugar and reducing sugars. This initial transformation seems to be due to internal causes and is largely independent of external conditions. Even at a temperature of 30° C. both cane sugar and reducing sugars accumulate during this initial period in excess of the quantity used in respiration, while during subsequent periods the quantity of reducing sugar diminishes at that temperature as a result of respiration. These initial changes seem to be associated with the cessation of the flow of materials from the vines.

In sweet potatoes stored at a temperature of 11.7° to 16.7° C., the moisture content remains fairly constant. There is a gradual disappearance of starch during the first of the season (October to March) and probably a re-formation of starch accompanied by a disappearance of cane sugar during the latter part of the season (March to June). The changes in reducing sugar are less marked than those in cane sugar. The changes in starch and cane sugar appear in a general way to be correlated with the seasonal changes in the temperature.

In sweet potatoes kept in cold storage (4° C.) there is a rapid disappearance of the starch and an accompanying increase in cane sugar. These changes do not attain a state of equilibrium at that temperature, as the sweet potatoes invariably rot by the action of fungi before the changes have reached their maximum. At both high and low temperatures cane sugar is the chief product formed by the conversion of starch in the sweet potato. The quantity of invert sugar in the root at any time is comparatively small.

LITERATURE CITED

APPLEMAN, C. O.

1914. Biochemical and physiological study of the rest period in the tubers of Solanum tuberosum. Md. Agr. Exp. Sta. Bul. 183, 226 p., 17 fig.

BARANETZKY, J.

1884. [Ueber die jährliche Periode der Stärkespeicherung in den Zweigen unserer Bäume.] *In* Bot. Centbl., Bd. 18, No. 18, p. 157.

BRYAN, H. A., GIVEN, A., and STRAUGHN, M. N.

1911. Extraction of grains and cattle foods for the determination of sugars: A comparison of the alcohol and the sodium carbonate digestions. U. S. Dept. of Agr., Bur. Chem. Circ. 71, 14 p.

FISCHER, A.
1891. Beiträge zur Physiologie der Holzgewächse. *In* Jahrb. Wiss. Bot., Bd. 22, p. 73–160.
GREBNITSKY, A.
1884. Über die jährliche Periode der Stärkespeicherung in den Zweigen unserer Bäume. *In* Bot. Centbl., Bd. 18, No. 18, p. 157.
HABERLANDT, GOTTLIEB.
1876. Untersuchungen über die Winterfärbung ausdauernder Blätter. *In* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Bd. 73, Abt. 1, p. 267–296.
HARRINGTON, H. H.
1895. Water and sugar in sweet potatoes as influenced by keeping. *In* Texas Agr. Exp. Sta. Bul. 36, p. 628–629.
LIDFORSS, B.
1907. Die Wintergrüne Flora. *In* Lunds Univ. Årssk., n. f. bd. 2, afd. 2, no. 13. 76 p., 4 pl.
MER, E.
1876. De la constitution et des fonctions des feuilles hivernales. *In* Bul. Soc. Bot. France, t. 23, p. 231–238.
MÜLLER, N. J. C.
1877. Vertheilung des Stärkerestes in der Strombahn. *In his* Botanische Untersuchungen, Bd. 1, p. 241–245. Heidelberg.
MÜLLER-THURGAU, H.
1882. Über Zuckeranhäufung in Pflanzentheilen in folge niederer Temperatur. *In* Landw. Jahrb., v. 11, p. 751–828.
ROSENBERG, O.
1896. Die Stärke der Pflanzen im Winter. *In* Bot. Centbl., Bd. 66, No. 24, p. 337–340. (For observations on the roots of trees, see Leclerc du Sablon, Sur la variation des réserves hydrocarbonées dans la tige et la racine des plantes ligneuses. *In* Compt. Rend. Acad. Sci. [Paris], t. 135, no. 20, p. 866–868. 1902.)
RUSSOW, E.
1884. [Über Tüpfelbildung und den Inhalt der parenchymatischen Elemente der Rinde.] *In* Sitzber. Naturf. Gesell. Univ. Dorpat, Bd. 6, p. 350–389.
1884. Über das Schwinden und Wiederauftreten der Stärke in der Rinde der einheimischen Holzgewächse. *In* Sitzber. Naturf. Gesell. Univ. Dorpat, Bd. 6, p. 492–494.
SCHULZ, ERNST.
1888. Über Reservestoffe in immergrünen Blättern. *In* Flora, Jahrg. 71, No. 14/15, p. 223–241, 248–258.
SHIVER, F. S.
1901. Sweet potato. II. Changes in composition on storing; III. Relative value of different methods of storing. *In* S. C. Agr. Exp. Sta. Bul. 63, p. 6–37.
WILEY, H. W., et al.
1908. Official and provisional methods of analysis, Association of Official Agricultural Chemists. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p.

PRELIMINARY AND MINOR PAPERS

THREE-CORNERED ALFALFA HOPPER

By V. L. Wildermuth,
Entomological Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology

INTRODUCTION

The small triangular insect of the hemipterous family Membracidae on which this paper is based was first noted and described as *Membracis festina* by Thomas Say in 1831 (1);[1] and in 1869 Stål (2, 3) referred it to the genus Stictocephala. Since that time it has frequently been noted by entomological writers, who usually merely mentioned its occurrence in a new locality or repeated what had already been observed. In 1888 this insect was first noted in literature as being injurious (4). However, the species was not generally considered of economic importance until the winter of 1910, when Prof. Herbert Osborn, in a paper (11) read before the American Association of Economic Entomologists, called attention to the economic habits of the genus Stictocephala and gave special attention to the species *S. festina* and its economic relation to alfalfa and clover.

Prof. Osborn in his paper stated that little or nothing was known of the life history and habits of the species. It is the purpose of this paper to give a report of the same, together with other related data, as collected by the writer, assisted by Messrs. R. N. Wilson and T. Scott Wilson at Tempe, Ariz., and by Mr. Edmund H. Gibson at Greenwood, Miss.

SPECIFIC IDENTITY OF THE THREE-CORNERED ALFALFA HOPPER

The name "three-cornered alfalfa hopper," adopted for this insect because it is the common term applied to it by farmers throughout areas of heavy infestation, is applicable to both Say's (1) *Stictocephala festina* and Van Duzee's (10) *Stictocephala festina*, var. *rufivitta*. On several occasions Mr. Otto Heidemann has determined a few specimens as *S. festina*, var. *rufivitta*, among material sent to the Bureau of Entomology for identification. As these were secured both by Mr. Gibson in Tennessee and Mississippi and by the writer in Arizona, one is led to believe that the species and the so-called variety occur rather generally together. Since Van Duzee (10) bases his description of the *rufivitta* variety upon male specimens only, and since only male specimens among hundreds examined have exhibited the determining character—namely, that "the dorsal carinæ are not evanescent before their point of meeting the

[1] Reference is made by number to "Literature cited," p. 362.

posterior carinæ," both the writer and Mr. Gibson, who has made many observations on this point, feel that this variety has been founded on too slender grounds.

Van Duzee's variety *angulata* has never been taken.

DISTRIBUTION

Osborn, in his paper (11) on the genus Stictocephala, points out that *S. festina* has a wide distribution and is found throughout the southern and southwestern United States. It is certain that in these sections it occurs in the greatest abundance, but its range is not limited to them. (See fig. 1.) Say (1) described the species from specimens secured in Florida. In 1889 Provancher (5) reported the species in Ottawa, Canada, and in 1890 Smith (6) gave New Jersey as a new locality. Later

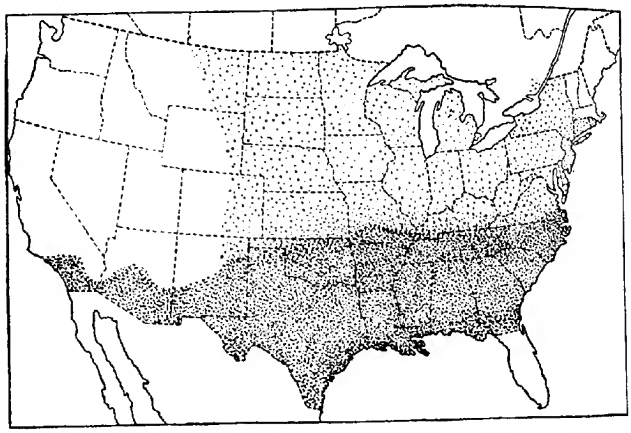


FIG. 1.—Map showing distribution of the three-cornered alfalfa hopper (*Stictocephala festina*) in the United States. The densely dotted area shows region of injurious infestation; the sparsely dotted area shows region of occurrence in limited numbers. Original.

than this (1894) F. W. Goding (8) gave the following localities: Virginia, Pennsylvania, Georgia, Florida, Missouri, Texas, Iowa, Montana, and Colorado (Riley); New York and Connecticut (Van Duzee); New Jersey (Smith); Canada (Provancher). That the species occurs in very limited numbers in the northern half of the United States is certain. Osborn found in his travels of 1909 and 1910 that *S. lutea* has a southern boundary agreeing quite well with the northern boundary of *S. festina*. The writer had the pleasure of examining alfalfa sweepings made by Mr. R. N. Wilson at 12 different localities in Colorado and Utah during the summer of 1911, and although several species of Membracidae were represented in the collections, not one specimen of *S. festina* was present.

The writer has observed the species in abundance throughout the Southwestern States, and found it injuring alfalfa (*Medicago sativa*) at Yuma, Tucson, Casa Grande, Tempe, Phoenix, Buckeye, and Glendale, Ariz., while Mr. R. N. Wilson reported it in alfalfa sweepings at Sacaton, Ariz., a region

isolated by a desert from any other cultivated area. Specimens were taken by the writer at Bard, Cal., another region remote from cultivated areas. Throughout the Imperial Valley in southern California the alfalfa hoppers were found in injurious abundance, while in Mexico, in the peninsula of Lower California, the pest was taken in numbers. In 1912 and 1913 Mr. Edmund H. Gibson found the species well distributed throughout the States of Mississippi and Tennessee and in several localities in Alabama, as well as at Atlanta, Ga.

EFFECT OF ALTITUDE ON DISTRIBUTION

It is quite interesting to note here that in Arizona and New Mexico the species is distinctly one inhabiting lower altitudes. During the summer of 1913 a great many observations were made on this point. The highest point, to the writer's knowledge, at which it has been taken is Fairbank, Ariz., where, at an altitude of 3,868 feet, on September 4, 1913, Mr. Harry Newton, then an agent of the Bureau of Entomology, found both nymphs and adults to be quite common on alfalfa. The writer made sweepings at Raton, Cimarron, and Las Vegas, N. Mex., all at an altitude above 5,000 feet, and while many alfalfa insects common in lower altitudes were taken, not a specimen of Stictocephala was secured. At Ute Park, Taos, Embudo, Bluewater, and Gallup, N. Mex., localities ranging in altitude from 5,000 to 8,000 feet, Mr. J. R. Sandige made sweepings from alfalfa and likewise failed to take a single specimen of Stictocephala, although it is known to occur in lower altitudes in the State. Possibly the most striking observations were those of Mr. R. N. Wilson, made while on a trip through Arizona for the express purpose of securing records on this species. He visited points varying in altitude from 2,000 to 7,000 feet, but never found the species above about 3,000 feet. His note, made on August 25, 1913, giving a summary of the trip, is as follows:

> The writer returned to-day from a trip over part of Arizona, including stops at Prescott, Camp Verde, Williams, Show Low, Pinetop, White River, and Gila River Valley points from Rice to Solomonsville and Miami. Special alfalfa sweepings were made at each of the above-mentioned places to determine whether or not *Stictocephala festina* occurred in that locality. The highest altitude at which the species was found was 3,000 feet, at Camp Verde. Altitudes varying from this to 7,000 feet were examined, but no trace of *S. festina* was found. When Rice was reached, where the altitude is only 2,500 feet, this species was again found in numbers, and all the way up the Gila River Valley to Solomonsville (altitude 2,985 feet) the Stictocephala were very common.

FOOD PLANTS

The alfalfa hopper lives on a great variety of food plants. Its general distribution and the fact that it is found in such isolated places under cultivation are doubtless due to the wide range of its food habits and probably, also, to the presence of native leguminous plants upon which, in all probability, it lives. Its favorite foods without a doubt belong to the legume family, for it is particularly fond of alfalfa, cowpeas (*Vigna sinensis*), and the various clovers, but it has also been found feeding upon trees, shrubs, herbs, and grasses.

The earliest recorded food plant is the tomato, which in 1888 was reported by Dr. Oemler (4) as being injured by this species. The next record we have is Prof. Cockerell's (9) in 1899, when he reported the hopper as feeding on alfalfa. He also mentions its occurrence on almond

trees, but does not say whether it was feeding thereon or not. Prof. Osborn (11) reported it in 1910 as feeding upon both alfalfa and clover. The writer has found the species feeding as well as breeding on Bermuda grass (*Capriola dactylon*), Johnson grass (*Sorghum halepense*), wheat (*Triticum* spp.), barley (*Hordeum sativum*), oats (*Avena sativa*), bur clover (*Medicago denticulata*), yellow sweet clover (*Melilotus officinalis*), and alfalfa, which, as has been stated, is its principal food plant.

Mr. T. Scott Wilson took specimens feeding on soy bean (*Glycine hispida*) at Sacaton, Ariz., and Mr. Edmund H. Gibson, besides reporting the species as feeding upon alfalfa, also finds it feeding upon vetch and *Hordeum murinum* at Tempe, Ariz., and upon red clover and cowpeas at Greenwood, Miss., and in fact doing its greatest damage to the last-named plant. Dr. A. W. Morrill, State Entomologist of Arizona, has found the insect feeding upon beans and in some instances proving a pest to that plant. Late in the season one finds the insect resting upon many varieties of plants, but whether feeding on all these is unknown. Mr. R. N. Wilson found it upon the following plants: Sunflower, upon which it was doubtless feeding; cocklebur; *Atriplex truncata; Erigeron canadensis* and *Erigeron* sp.; mesquite and cottonwood, feeding on the former; *Sporobolus airoides*, and *Trichlaris mendocina*.

DESCRIPTION OF THE THREE-CORNERED ALFALFA HOPPER

THE ADULT

The adults (Pl. XLIII, fig. 1) are about 6.16 mm. long and light green in color. The accompanying table of measurements (Table I) made by Mr. Gibson shows that the males are slightly smaller than the females.

TABLE I.—*Length of live adults of the three-cornered alfalfa hopper*

Specimen No.	Length of—	
	Male.	Female.
	Mm.	*Mm.*
1	6.0	6.0
2	6.4	6.3
3	6.1	6.3
4	5.9	6.0
5	6.0	6.6
Average length [a]	6.8	6.24

[a] Average length of 10 adults, 5 males and 5 females, is 6.16 mm.

The males have a reddish line down the dorsum of the prothoracic shield. This marking, being absent in the female, is a sex character by which mature males and females are quite readily distinguishable. The insects are triangular in shape, presenting a broad solid aspect when viewed from the front (Pl. XLIII, fig. 1, *b*). The following original description, made by Thomas Say (1) in 1831, was evidently made from male specimens, because, as is mentioned above, the females do not have the "carina tinged with rufous."

Thorax with a subacute line each side before, meeting behind the middle. Inhabits Florida.

Body yellowish-green: thorax unarmed, carinate behind; at tip attenuated, subulate and complying with the general curvature; each side before a carinate line, meeting together at the carina behind the middle, with the carina tinged with rufous; front of the thorax not altogether flat, but a little convex; hemelytra, three terminal cellules unequal; the two costal ones equal, as broad as long; the inner one not obviously larger than the others together, somewhat longer than broad. Length to tip of hemelytra one fifth of inch. The lateral prominent lines of the unarmed thorax, separate this species from all those I have described excepting goniphera, which, meet before the middle of the length of the back.

THE EGG

The egg (Pl. XLIII, fig. 2, *b*) is about 1 mm. (0.9 to 1.3 mm.) long and 0.35 mm. (0.25 to 0.4 mm.) in diameter. It is white, rather oblong, slightly larger at one end, and with a greater curve on one side. The surface is smooth, except a portion on the larger end which is regularly covered with small papillæ.

THE NYMPH

The nymphs are the same general shape as the adults, but instead of having the prothoracic shield as a body covering, they are regularly covered with prominent projections, spines, and hairs. There is one dorsal pair of these projections on the head, four pairs on the thorax, and seven pairs on the abdomen, the posterior pair on the anal segment being much reduced in size.

Their general color is as follows: Head and thorax very light straw. Eyes with margin white and center cologne earth. Antennæ white. Thorax with a regular cologne-earth patch on each side, widest on the mesothorax, where it reaches the darkest shade. Legs white, except tip of last tarsal joint, which is dark brown. Abdomen white, approaching light green, owing to food material within, with the irregular dark spot of the thorax extending narrowly across the first segment, widening greatly on segments 2, 3, and 4, and showing only on the posterior margin of the fifth, being widest on the posterior margin of segments 3 and 4. Anal segment light-straw color at extreme end.

The different nymphal stages, of which there are five, are the same in general appearance, except that the main dorsal projections in the first stage have only one subspine, while in the second and remaining stages there are numerous branches. A second difference is the growth posteriorly of the prothoracic shield and the appearance of wing pads in the last three stages. These two differences, with the increase in the size of the body and the general darkening of colors in each successive stage, enable one to recognize any of the different stages.

DESCRIPTION OF INDIVIDUAL STAGES

STAGE I (Pl. XLIII, fig. 2, *a*).—Length, 1.6 mm. (1.4 to 1.7 mm.), average of 10 specimens. Head, thorax, abdomen, and all appendages pale when first born. After feeding the abdomen takes on straw color and the cologne-earth patch of the thorax becomes faintly visible. Eyes white. Twelve pairs of dorsal hairlike projections with one upright spine. One pair on head, four on thorax (two on prothorax, one each on mesothorax and metathorax), and seven on abdomen. Spines colorless, pale. Body regularly but sparingly covered with spines, conspicuous and large compared to size of body.

STAGE II (Pl. XLIII, fig. 3).—Length, 2.1 mm. (1.9 to 2.5 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages light straw colored. The cologne-earth patch of the thorax becomes more pronounced and extends back onto the abdomen. Eyes in this and succeeding stages with white margin and brown center. The dorsal projections have become fleshy and bear several lateral spines, the upright spine

being reduced in length. Other body spines much more numerous than in first stage, but reduced in proportionate size.

STAGE III (Pl. XLIII, fig. 4).—Length, 2.9 mm. (2.6 to 3 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages dark straw color, cologne-earth patch in some specimens especially pronounced. Dorsal projections more fleshy and containing a greater number of lateral spines. Prothoracic shield beginning to develop. Wing pads faintly visible.

STAGE IV (Pl. XLIII, fig. 5).—Length, 3.8 mm. (3.5 to 4.1 mm.), average of 10 specimens. Head, thorax, abdomen, and appendages greenish straw color. Dark patch on thorax and abdomen becoming dark brown, almost black. The color varies greatly, however, some specimens being light green and others very dark throughout the stage. Dorsal projections in this and fifth stage quite fleshy, lateral spines numerous. Prothoracic shield with posterior projection extending nearly to end of thorax. Wing pads clearly defined.

STAGE V (Pl. XLIII, fig. 6).—Length, 4.8 mm. (4.5 to 5 mm.), average of 10 specimens. Color same as in Stage IV, about the only difference between this stage and Stage IV being the enlarged size. Point of prothoracic shield extending over the first segment of the abdomen and wing pads extending to posterior part of second abdominal segment.

LIFE HISTORY AND HABITS

The observations on the three-cornered alfalfa hopper have been carried through two years and parts of two others at Tempe, Ariz., and through one entire year at Greenwood, Miss. The results, therefore, have been secured under widely differing conditions, the former place being in a hot, semiarid country with an annual rainfall of about 8 inches, while the latter is in a warm, humid country with an average annual rainfall of nearly 50 inches.

In the Salt River Valley of Arizona much difficulty was experienced in securing life-history records during the months of June, July, and August, because of the excessive heat. In order to have the specimens under close observation, it was, of course, necessary to confine them in cages under more or less artificial conditions, and under such conditions the death rate among nymphs was very high. The combined lengths of the egg and nymphal stages under Arizona conditions varied with the temperature, being from 35 to 114 days, with an average of about 50 days for all conditions. In Mississippi Mr. Gibson found that a much shorter period was required. Here the variation, as shown by observations on a much smaller number of specimens, was from 26 to 37 days. Records of a larger number of specimens would doubtless have given a wider variation.

EGG STAGE

In Arizona the egg stage varies from a minimum of 12 days to a maximum of 41 days, this variation depending upon the prevailing temperature. The average for all records is 22 days. As may be seen in Table II, during an average mean temperature of 59° F. the time required for incubation varied from 33 to 43 days. At a mean temperature of 63° the variation was from 23 to 30 days, while with a mean temperature of about 85° the eggs hatched in a period ranging from 12 to 17 days.

TABLE II.—*Length of the egg stage of the three-cornered alfalfa hopper at Tempe, Ariz., in 1912; host, alfalfa*

Cage No.	Eggs laid.		Eggs hatched.		Length of incubation.	Temperature.
	Date.	Number.	Date.	Number.		
					Days.	°*F.*
1[a]	Feb. 6	2	Mar. 7	2	30	.....
5[a]	6	1	7	1	30	.....
9	Mar. 19	[b] Many.	Apr. 22	2	33	[c] 59
			23	1	34	
			25	1	36	
			May 1	6	43	
10	22	Many.	Apr. 25	2	34	[c] 59
			26	4	35	
			27	3	36	
			May 1	3	40	
			2	4	41	
16	Apr. 9	Many.	3	1	24	63
			5	1	26	
17	10	Many.	3	1	23	63
			4	0	.....	
			5	2	25	
			6	4	26	
			7	2	27	
			8	3	28	
			9	1	29	
			10	2	30	
24	May 24	Many.	June 5	28	13	83
			6	25	14	
			7	49	15	
			8	12	16	
25	25	Many.	6	3	12	84
			7	13	13	
			8	4	14	
			10	1	16	
37	June 21	Many.	July 6	7	15	85
			7	3	16	
			8	2	17	
38	July 5	Many.	20	0	15	87
			21	3	16	
			22	2	17	

[a] Influenced by artificial heat.
[b] No way of getting exact count without disturbing the eggs.
[c] Average mean temperature.

At Greenwood, Miss., Mr. Gibson was able to get eggs to hatch in the remarkably short time of four days. On June 30 eggs were deposited in cowpea stems, and on July 3 several had hatched. The writer is unable to surmise the reason for this short duration of the incubation period. The temperature, although no records are available, could hardly have been higher than that recorded during July at Tempe. The amount of humidity may have had something to do with the hasty incubation; then, too, the different host plant, all records from Tempe having been made on alfalfa, may have been a factor in lessening the period. Table III gives the results of Mr. Gibson's experiments to determine the length of the egg stage.

TABLE III.—*Length of the egg stage of the three-cornered alfalfa hopper at Greenwood, Miss.; host, cowpeas*

Date of oviposition of adults.	Date of egg hatching.	Length of egg stage.	Number of eggs.
		Days.	
June 30	July 3	4	10
Sept. 1	Sept. 8	7	Many
3	8	5	8

OVIPOSITION

IN ALFALFA.—The egg is deposited beneath the epidermis through a long slit made in the stem of alfalfa by the female with her ovipositor. This slit is often several times the length of the egg. Measurements of a large number of slits displayed a variation in length of from 0.75 to 2.25 mm. The egg is placed either just below or to one side of this puncture, and occasionally, instead of being just under the epidermis, an egg may be found shoved deep within the plant tissues, even to the center of the stem or beyond. Usually only one egg is deposited through a single opening, but sometimes two or more are placed together. Quite often, however, a great many slits are grouped side by side and the eggs laid singly but giving the appearance of having been bunched through the same opening. When this is the case, a large scar is made, and the place of oviposition would be quite noticeable if it were not for the fact that it usually occurs back of a sheath leaf or at the surface of the ground, where it is partially hidden. Females have been found with their abdomen extended down the stem of the plant and below the surface of the ground, and subsequently eggs have been found in the stems at such places. It has been observed that eggs are usually laid at night or early in the morning. These observations were made, however, during extremely warm weather; during cold weather the females would probably pick out the warmer part of the day to display their activities and thus avoid the minimum temperature, as they doubtless avoid extreme temperature.

IN COWPEAS.—The method of oviposition in cowpea stems is considerably different from that in alfalfa, the texture of the cowpea plant evidently making possible the placing of a great many eggs in the stem through one opening. Mr. Gibson has found that the eggs are always laid in groups and in his field notes quite aptly refers to these places of oviposition as egg pockets. He has observed from 1 to 12 eggs in a pocket. A count of the eggs in six pockets showed respectively 6, 4, 12, 2, 3, and 5 to the pocket. Following oviposition, in about one-third of these pockets a gall formation develops. From their appearance these must be similar to the galls which develop on alfalfa stems following ringing and which are described in the paragraph on alfalfa injury. These naturally give the egg pockets a distinctive and peculiar appearance. They are often as large as the stem itself, sometimes as much as one-fourth of an inch in diameter, and well show the efforts of the plant toward the healing of the injured part. Mr. Gibson thinks that these galls are due to an overproduction of epidermal cells caused by the physiological stimulus given to the plant by the injury, and states that they are of about the same texture and hardness

as the plant stem itself. It seems probable that the eggs in pockets where these galls have developed may be so interfered with that they can not incubate, but no definite observations were made on this point.

NYMPHAL PERIOD

The nymphal period comprises five stages, with a total length of from 22 to 69 days, depending upon the prevailing temperature. As is shown in Table IV, during the cooler spring month of March the total length varied from 42 to 69 days, while in the hot month of July the variation was from 22 to 37 days. The length of the different stages was found to be very unequal, the last two being found to average the longest, while the fifth might be prolonged almost indefinitely, provided food or other conditions were not right. This in itself is an important point, for the species would be able to survive for some time on only a minimum of food. There was found to be very little relation between the length of the periods and the sex of the individual.

TABLE IV.—*Lengths of the nymphal stages of the three-cornered alfalfa hopper at Tempe, Ariz., in 1912 and 1913*

Cage No.	Date of hatching of egg.	Date of first molt.	Length of Stage I.	Date of second molt.	Length of Stage II.	Date of third molt.	Length of Stage III.	Date of fourth molt.	Length of Stage IV.	Date of fifth molt.	Length of Stage V.	Total length of nymphal period.		Mean average temperature.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*	*Sex.*	*Days.*	*°F.*
	1912.													
1 a	Mar. 7	Mar. 17	10	Mar. 25	8	Apr. 4	10	Apr. 15	11	Apr. 28	13	♂	42	....
3 a	7	17	10	24	7	3	10	14	11	30	16	....	44	....
4	7	19	12	Apr. 3	15	11	8	25	14	May 13	18	♀	67	61
4	7	20	13	3	14	11	8	25	14	13	18	♀	67	61
5	7	19	12	Mar. 29	10	8	10	19	11	15	26	♀	69	61
6	8	20	12	27	7	17	21	29	12	15	16	♂	68	61
12	27	Apr. 7	11	Apr. 14	7	21	7	29	8	17	18	♀	50	65+
15	27	7	11	15	8	21	7	29	8	25	25	♂	57	65+
21	May 1	May 9	8	May 14	5	May 20	6	June 1	12	June 12	11	♀	42	76+
22	1	7	6	13	6	19	6	May 29	10	14	16	♂	44	76
30	June 8	June 15	7	June 20	5	June 24	4	June 29	5	July 9	11	♂	32	84
30	8	16	8	21	5	25	4	July 1	5	10	9	♂	33	84
38	July 22	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	Aug. 28	(b)	2 ♂	37	86
39	6	(c)	(c)	(c)	(c)	(c)	(c)	(c)	(c)	8	(c)	♀ and 2 ♂	33	86
41	21	(d)	(d)	(d)	(d)	(d)	(d)	(d)	(d)	Aug. 22 and 23.	(d)	♂ and ♀	32 and 33	86
	1913.													
49	Apr. 12	Apr. 18	6	Apr. 24	6	Apr. 29	5	May 7	8	May 25	18	....	43	69
50	12	17	5	24	7	29	5	9	10	27	18	....	45	69
52	May 1	May 8	7	May 12	4	May 17	5	(e)	(e)	June 9	22	♂	39	73
53	1	8	7	13	5	18	5	27	9	9	13	....	39	73
56	1	7	6	12	5	15	3	20	5	1	12	....	31	71
57	1	8	7	13	5	19	6	24	5	8	15	♀	38	73
59	1	8	7	12	4	17	5	24	7	8	15	♀	38	73
60	2	8	6	12	4	(e)	(e)	21	9	May 28	7	....	26	71
61	2	7	5	12	5	May 15	3	22	7	June 6	15	♀	35	73
62	2	8	7	12	4	17	5	24	7	14	21	♀	44	74
63	5	10	5	14	4	19	5	25	6	8	14	♂	34	72
67	5	9	4	13	4	18	5	26	8	14	19	♀	40	73
72	7	11	4	17	6	23	6	30	7	8	9	♂	32	75
73	7	17	10	21	4	27	6	(e)	(e)	8	12	♂	32	75
84 f	July 1	July 6	5	July 9	3	July 13	4	July 17	4	July 25	8	♀	25	87−
85 f	3	7	4	10	3	13	3	17	4	26	9	♂	23	86
86 f	1	5	4	8	3	12	4	16	4	23	7	♂	23	87+
87 f	1	5	4	9	4	13	4	18	5	27	9	♂	27	86

89 *f*	1	5	4	9	4	12	3	16	4	22	6	♂♀	22	87+
90 *f*	2	7	5	10	5	14	4	18	4	28	10		26	87−
91 *f*	2	7	5	10	3	14	4	19	5	30	11		28	85+
Average	....	....	7.2	....	5.8	....	6	....	7.66	....	14.25	....	38.75	....
Maximum	....	....	13	....	15	....	21	....	14	....	26	....	....	....
Minimum	....	....	4	....	3	....	3	....	4	....	7	....	....	....

a Influenced by artificial temperature.
b 2 specimens; records missing.
c 3 specimens; records missing.
d 2 specimens; records missing.
e Records missing.
f Records by T. Scott Wilson.

It is to be noted that in the third stage the 21-day maximum was observed in only one specimen, and this seems to be an extreme one, as the next highest maximum was only 10 days.

Looking at the Greenwood (Miss.) records for the nymphal periods, one finds not nearly as much variation between these and the records for Tempe, Ariz., as was exhibited in the incubation records for the two places. The nymphal period required from 22 to 30 days for completion with cowpeas as a host plant.

TABLE V.—*Length of nymphal period of the three-cornered alfalfa hopper at Greenwood, Miss.*

Date of emergence from egg.	Date of last molt.	Length of period.
		Days.
July 3	Aug. 3	30
Aug. 8	Sept. 3	26
Sept. 8	30	22
8	Oct. 6	28

HABITS OF THE NYMPHS

HATCHING.—The egg in hatching splits across one end and about one-fifth of the way down one side, and the nymph wriggles its way out. Its legs spread and in a few minutes it begins feeding. Two specimens were timed and one required 18 and the other 28 minutes to complete the process.

PROTECTION.—If left alone, the first-stage nymphs are very quiet and slow of movement, feeding in almost the same spot for days. As soon as approached by any object, they hastily place themselves on the other side of the plant and out of harm's way. The older nymphs are quite active and along with the younger exhibit a peculiar protective habit. If approached by an enemy or a supposed enemy, as the point of a camel's-hair brush, they throw the point of the abdomen toward the object and voiding a large bubble of watery excrement, explode it in the face of the enemy and then hastily move to the other side of the plant. In teasing a nymph in order to get it to display this habit the writer has cautiously moved the point of a lead pencil at the head of the nymph, and in trying to project the anal segment towards the pencil the nymph would nearly lose its footing. This habit is probably of considerable benefit as a protection and along with the horny appearance of the nymph doubtless furnishes immunity from many a hungry foe.

MOLTING.—The process of molting is interesting. As observed in two specimens it required 48 minutes for the one and 32 minutes for the other. Most of this time was occupied in getting a split started in the thorax. After the split was once started, the actual time required for the insects to wriggle out was 2 and 5 minutes, respectively. The description of the action is taken from the writer's original notes, made on March 20, 1912.

Just previous to molting, the skin becomes very tight and rigid, owing, of course, to pressure from within. The abdomen appears like an overinflated football bladder. The specimen, becoming quiet, forces its proboscis firmly into the stem, and using this as a pivot, with its legs to assist, it begins various body movements, such as straightening out its head, waving its abdomen up and down and, alternately with this, hunching the thorax upward, then resting a brief moment, whereupon the same

process is repeated. This was continued for three-quarters of an hour and then, after a minute's rest, with one final effort an opening was split on the dorsum of the entire thorax and head and the delicate white insect began to appear. The spines on the thorax were first pulled out, and then the insect continued its wriggling and gradually the abdomen was pulled from the old abdominal skin, the larva all the time working itself forward over the cast skin of the head. The legs were not entirely withdrawn until after the last segment of the abdomen was freed. The insect, then crawling the rest of the way over the head of the exuvia, came to rest on the plant just ahead of the cast skin. There it remained resting for 30 minutes, during which time the newly exposed tissue was becoming hard and firm and accustomed to the surrounding atmospheric conditions, after which the insect began feeding.

THE ADULT STAGE

The adults are strong, quick flyers. They are wary and, like the young, upon the approach of danger hastily move to the opposite side of a plant; then, as the enemy comes closer, with a spring they are off—how swiftly can only be appreciated by one who has been unexpectedly hit on the face or in the eye by an alfalfa hopper.

As observed in the cages kept for the purpose of determining the number of generations, the males are more numerous than the females, being in the proportion of 4 to 3. The female, after issuing from the last nymphal stage, requires from 8 to 10 days to complete her development. During this time she is feeding, and at the end of the period copulation takes place. A few days thereafter oviposition begins. The males die shortly after copulation, while the females lay eggs for a considerable period. Four hibernating females were placed in a cage on February 13, and on February 26 several eggs were laid; oviposition was continued until May 5, the females dying soon thereafter. Thus there was an egg-laying period of 70 days. A maximum of only 50 eggs was secured from a single female. The four mentioned above laid a total of 129 eggs, or an average of 32 each. It is possible, however, that these may have deposited eggs previous to entering hibernation. Eight other females deposited from 7 to 19 each. These numbers all seem small for maximums, but they were secured under unnatural cage conditions. Without much doubt a larger number than this, possibly as many as a hundred, are deposited by single females, when they are free and unhampered, as in the field.

SEASONAL HISTORY

HIBERNATION

The seasonal history of this species varies during different years, the variation quite naturally being due to the climatic conditions, especially the minimum temperature of any particular year. This variation appears largely during the winter months, when the species is supposed to be hibernating. At Tempe, Ariz., during a mild winter, such as the last one (1913–14), the species does not hibernate at all in the adult stage. Adult males and females were taken feeding on alfalfa every week during December, January, and February of last winter. Mr. Gibson, who made the January and February observations at Tempe, discovered that eggs deposited in the late fall months hatched on warm days, but the nymphs were usually killed during the cold nights following. During winters when the minimum temperatures are much lower the species goes into hibernation both as eggs and adults. During the winter of 1912–13 such conditions as those just mentioned were noted,

and because of the variation in minimum temperatures existing between the winter of 1912–13 and that of 1913–14 and its relation to hibernation, Table VI, showing the minimum temperatures, is given.

TABLE VI.—*Average minimum temperatures at Tempe, Ariz., during the winters of 1912–13 and 1913–14*

Period.	1912–13	1913–14
	°F.	°F.
Dec. 1 to 10	39	35
11 to 20	33	40
21 to 31	27.5	38
Jan. 1 to 11	24.5	40.3
10 to 20	32.7	43.5
21 to 31	33.7	40
Feb. 1 to 10	39.2	32.3
11 to 21	35.5	45.2
21 to 28	38	40
Average minimum for 3 months	33.6	39.4
Actual lowest temperature	12	29

While it will be noted in Table VI that there was a difference of nearly 6 degrees in the average minimum of the three months, December 1912, and January and February, 1913, as compared with the same three months of 1913–14, yet the most striking difference and the one that influenced hibernation is noted between December 11 and February 1 of the two years. For the former winter, the one during which the species hibernated, the average minimum from December 11 to January 31 was 30.3° F., and the actual lowest was 12° F., as against an average minimum of 40.3° F. and an actual lowest of 29° F. for the same period of the winter 1913–14, during which time the species was continually active.

During the winter of 1912–13 the hibernating period lasted about two months. Just how long it may last any other winter will depend upon the temperature; if, as was shown to be the case last winter, the temperature remains high enough, the insect will not go into hibernation. At Tempe the adults have been found particularly abundant at the base of bunch grass (*Sporobolus airoides*) and they are also found hiding below rubbish, leaves, etc., at the base of plants such as will provide them with green food on the first warm days of spring.

In Mississippi Mr. Gibson has found that the hibernating period lasts from December until March and that the chief protection for the dormant adults consists of bunches of *Andropogon* spp., in the clumps of which he has counted as many as 63 adults. At Nashville, Tenn., he has observed hibernating adults active by March 11.

SUMMER ACTIVITY

In Arizona hibernating adults that come forth during the first part of February deposit eggs soon thereafter, and during the latter part of March, April, and the first part of May die off. Young of the first generation, whether they come from overwintering eggs or from eggs of hibernating adults, appear during the month of March. There are from three

to four generations annually. During 1912 the writer observed three and a partial fourth, and during 1913 Mr. T. Scott Wilson observed the same number. The species reaches its greatest numbers during September, when adults of the third generation are appearing. Immediately following the first of November the adults begin disappearing quite rapidly. Many doubtless deposit their complement of eggs and die a natural death, others are killed by the approach of cold weather, and the rest go into hibernation. Of the immense numbers that go into hibernation but few appear in the spring. This heavy mortality is doubtless due to the varying temperature. A week of warm days appears. The insects, thinking spring has come, desert their protected places and begin feeding. Then, if the night temperature suddenly drops to freezing or below, a great many of them succumb.

The actual dates for the different generations as observed in cages are given in Table VII. Under field conditions it is quite probable that there would be considerable variation from these dates, but they can be considered as an average for the different conditions.

TABLE VII.—*Periods of generations of the three-cornered alfalfa hopper in Arizona in 1912 and 1913*

Generation.	1912	1913
First generation	Feb. 6 to June 10	Feb. 3 to May 28.
Second generation	June 10 to Aug. 7	May 28 to Aug. 1.
Third generation	Aug. 7 to Oct. 10	Aug. 1 to Oct. 1.
Fourth generation	Oct. 10; no eggs	Oct. 1; nymphs in November.

DAMAGE TO ALFALFA

INJURY TO THE PLANT

The damage to alfalfa and other plants comes as a result of the sucking up of the plant juices for food by the adults and nymphs. The sharp-pointed proboscis-like mouthparts or beak is thrust into the plant and the juice extracted, leaving the plant wilting and often in a dying condition. Both the adults and the young have two methods of feeding. One is a promiscuous puncturing of the stems, while the other is the puncturing in a regular and continuous line which takes the form of a ring or girdle around the stem (Pl. XLIII, fig. 7, *a*). At first it was suspected that this girdling had something to do with egg deposition, since the eggs, being deposited below the girdle which had stopped the circulation of plant fluids, were safe from injury by plant growth. Soon, however, it was noticed that nymphs were more often responsible for the ringing than adults and that girdling from adults had no relation whatever to oviposition.

It is from these girdling punctures that the greatest damage results; for in addition to the loss of plant juices, the stems are weakened, a gall (Pl. XLIII, fig. 7, *b*) usually develops, circulation is cut off from the upper portion of the plant, and a great many of the plants break off, become yellow, and die. It is interesting to note here that the nymphs do more damage than the adults. They seem to be much more hearty

feeders, and, being more sedentary, their feeding is more nearly restricted to a definite area or ring, and with this concentration of work the effect on the plant is more pronounced.

The gall following the girdling of the stem, shown in Plate XLIII, figure 6, *b*, is also quite detrimental to the plant. It is an effort on the part of the plant to mend the injury. There is always a thickened area in the epidermis both above and below the ring, and often this takes peculiar shapes. At one time the swellings will take the shape of globules larger in diameter than the stem itself; at other times a rootlike projection, often half an inch in length, will shoot out; and nearly always, sooner or later, under the pressure of wind or other external influence, the plant will break off and be of little value as food. The more tender stems are always chosen by the insects in preference to the older and more fibrous ones, and thus the maximum of food is found with the least labor. During cool days and cooler weather the feeding is done close to the ground; during warmer weather the species feeds high up on the plant and in the extreme heat of the summer it feeds on the shady side of the stem.

INJURY TO THE CROP

The damage to alfalfa, while not as serious as that caused by some other alfalfa pests, is considerable. To the casual observer it does not appear to be so heavy, chiefly because nothing is seen to be devoured, as in the case of lepidopterous larvæ; and yet, because of the great numbers appearing in alfalfa in the late summer months, farmers have often complained of the hoppers in their fields and have imagined that they were doing damage which in reality was due to larvæ of the yellow alfalfa butterfly (*Eurymus eurytheme*). As has been noted, during the latter part of August and continuing through September that species, as well as a jassid, *Empoasca mali* Le B., attains immense numbers and flies in great swarms before one in an alfalfa field. At this period of the year the alfalfa is fibrous, lacks succulency, and the growth is neither heavy nor thrifty. The hot weather is usually blamed for all this, but the fact is that a considerable percentage of the injury is due to the action of these insects. With dozens of hoppers feeding upon every stem and hundreds upon every plant, all sucking the plant juices, checking plant growth, and girdling many stems, causing them to shrivel and possibly to die or even break off, it is no wonder that the alfalfa looks sickly and is of slow growth during these months. On September 10, 1912, the writer made the following note:

To-day by motor cycle I went to Chandler, Ariz., to inspect an alfalfa field on Mr. Childs's ranch, which was reported as being "killed off" by insects. Upon reaching the field, I found that the alfalfa was in bad condition. The stems were so scarred from the feeding punctures of *Stictocephala festina* and jassids that they presented a sticky, sickly appearance, and the stems were dry and shrunken so as to be pliable to the touch and not solid and rigid, as they should be. A great many fields to the southeast of Tempe show this damage to a greater or less extent.

From the notes of Mr. T. Scott Wilson I copy the following:

Tempe, Ariz., September 26, 1913. Stictocephala are very numerous now around Tempe. They are doing a great amount of damage, more than at any time this year. Many alfalfa stalks are completely girdled near the ground and will break off very easily at this ring where the bug has sucked the juice from the stalk. Some have a great many small spots scattered along the stalk where the insects have fed. Other stalks have new branches starting up just below the girdled place, and some are green below this ring and yellow above it. There isn't any doubt (in the writer's mind) that

this is a serious pest to late summer crops. The insects are so thick at present that when a man is walking through alfalfa they fly into his face and swarm ahead of him like bees.

During the early part of September, 1914, several complaints were received from southern Virginia by the United States Department of Agriculture of serious damage to alfalfa by the insect under discussion. One such infestation occurred at the county experiment station at Williamsburg, Va. In this case Mr. R. P. Cocke reported that fully 95 per cent of the plants were seriously affected by the characteristic girdling of the hopper. Specimens of the insect were sent to the Department for identification and found to be the three-cornered alfalfa hopper in its fourth nymphal instar.

DAMAGE TO PLANTS OTHER THAN ALFALFA

In Mississippi Mr. Gibson has found that this hopper does as much damage to cowpeas as it does to alfalfa, or more. He finds that the greatest damage comes when the cowpeas are small, possibly only two or four leaves having developed. In this case, when the plant is girdled it can not so well overcome the damage and usually wilts down immediately. Often as many as 15 nymphs would congregate on one cowpea plant and soon sap its life. One of the serious causes of injury to cowpeas is the oviposition of the females. As has been stated elsewhere in this paper, the eggs are laid in pockets in the stems of the cowpeas, and around these pockets galls often develop. The scars resulting from such action are often so large and so abundant—as many as eight on a single small plant—that the plant is greatly retarded in growth and may break off or die.

Dr. A. W. Morrill, State Entomologist of Arizona, has told the writer that in their work with bean insects they have discovered *Stictocephala festina* in large numbers on beans and probably doing quite a bit of damage. The writer has made no observation of the pest on these plants.

Although a great many other plants are fed upon by this insect, none of them seems to be greatly damaged. While Dr. Oemler, in 1887, reported (4) damage to tomato plants, there seems to be no record since that time of any damage to that crop, and the species has certainly not become of great importance in relation to tomato culture.

NATURAL ENEMIES OF THE ALFALFA HOPPER

During the study of the three-cornered alfalfa hopper as an alfalfa pest it has been shown that it suffers in a remarkably small degree from natural enemies. Prof. T. D. A. Cockerell in 1899 observed (9) a spider, *Argiope transversa* Emerton, feeding on the alfalfa hopper at Phoenix, Ariz. The writer has also noticed remains of the insect in spider webs in alfalfa fields, but these do not exert any remarkable influence in reducing the numbers of the pest. Likewise, the harvester ant (*Pogonomyrmex barbatus* Smith) has been noticed by both the writer and Mr. Gibson carrying individuals of *Stictocephala festina*, but these must have been dead or disabled before capture by the ants. A small red predaceous mite, *Erythraeus* sp., was found feeding upon the eggs, choosing those with the outer end protruding above the plant tissues. Dr. O. C. Bartlett, Assistant State Entomologist of Arizona, informs the writer that in his work with this insect as a bean pest he has reared large numbers

of egg parasites from eggs deposited in the stems of bean plants and expects to publish a report concerning the matter in the near future. The writer, however, has never noted egg parasites issuing from eggs laid in alfalfa stems.

Dr. A. K. Fisher, of the Bureau of Biological Survey, United States Department of Agriculture, informs the writer that they have found stomachs of nighthawks to contain specimens of Stictocephala which these birds must have taken quite early in the evening. Messrs. R. N. and T. Scott Wilson, during September and October, 1913, killed 31 birds that were visiting alfalfa fields, and 10 of these had from one to four adults of *Stictocephala festina* in their crops. Table VIII shows the results of an examination of the stomachs of these birds. The birds were determined by Mr. Frank W. Rogers, State game warden of Arizona.

TABLE VIII.—*Results of the examination of the stomachs of 31 birds killed in alfalfa fields, showing feeding on Stictocephala festina*

Date.	Kind of bird.	Number of stomachs examined.	Number of *Stictocephala* sp. in each stomach.
1913.			
Sept. 12	Killdeer (*Oxyechus vociferus*)	1	1
12	Black phoebe (*Sayornis nigricans*)	1	2
12	Gambel's sparrow (*Zonotrichia leucophrys gambeli*)	6	0
12	Cassin's kingbird (*Tyrannus vociferans*)	1	0
12	Western mourning dove (*Zenaidura macroura marginella*)	1	0
Oct. 24	....do	2	0
21	Inca dove (*Scardafella inca*)	1	0
21	Western meadow lark (*Sturnella neglecta*)	1	0
24	....do	2	0
21	Sonoran redwing (*Agelaius phoeniceus sonoriensis*)	3	0, 4, 0
24	....do	12	0, 3, 1, 0, 0, 1, 2, 1, 0, 1, 0, 1

The same investigators killed 19 toads but only found three of these to have *Stictocephala festina* in their stomachs. One stomach contained one nymph and three adults, while two others contained one nymph and one adult, respectively. One would think that toads might feed upon the nymphs to a considerable extent, but the dissections of these 19 stomachs seem to prove the contrary.

PREVENTIVE MEASURES

The great problem is how to control the species. While good may be accomplished by any one of several methods, yet so far no way has been found for entirely controlling the pest. Prof. T. D. A. Cockerell (9) in 1899 suggested that a hopperdozer might be used successfully, but several attempts made during the fall of 1913 by Messrs. R. N. and T. Scott Wilson in which hopperdozers of different forms were used were all unsuccessful. A device with merely the upright canvas back of the oil pan caught only a very small percentage of the alfalfa hoppers. They are so quick and active that they get away without even touching the machine. When a forward projection of cloth was arranged so that the hoppers could not get over the already high back, a few more were taken, but the majority would fly out ahead and to one side, so that a hopperdozer seemed altogether impracticable.

Prof. Osborn (11) suggested timing the removal of the crops so as to destroy the eggs. While it is a certainty that many eggs are destroyed in this way, yet the fact that a large percentage is laid close to the ground and below the point above which they would be removed by the cutting process precludes any possibility of this method being successful.

In several instances fields that were pastured were found to be less infested, but this may have been a coincidence, and at any rate could not be utilized as a method of controlling the pest.

The one practice that will bring about a considerable reduction of the insects is clean methods of farming. When the time comes that each and every farmer is cultivating only as much land as he has means to handle properly—and by the term "handle properly" is meant the tilling of his land in such a way that the maximum returns per acre will be secured—then and then only will insect devastations be reduced to a minimum. The alfalfa hopper can be greatly reduced by just such handling, which must include the eradication of weeds, brush, bunches of wild grass, rubbish, etc., along fences, ditch banks, and other places. The fact that the alfalfa hopper is found during hibernation in places where it is protected from cold and from exposure to its enemies shows that a great many wintering adults may be eliminated by cleaning up such hiding places.

SUMMARY

The three-cornered alfalfa hopper (*Stictocephala festina*) is an insect of economic importance to alfalfa crops in the irrigated valleys of the southwestern United States and to alfalfa and cowpeas in the Southern States.

Injury is due to the sucking of plant juices by both adults and larvæ and the development of a feeding scar which often takes the form of a ring or girdle and which is usually accompanied by a gall formation.

Plants of the legume family constitute the favorite food.

The eggs are deposited in the stems of the food plants, usually back of the sheath leaves or below the surface of the ground. In cowpeas the eggs are deposited in pockets on the stems.

The egg period in Arizona occupies from 12 to 41 days and the five stages of the nymphal period from 22 to 69 days. The average combined length for both periods is about 50 days.

In southern Arizona there are four generations annually and during extremely mild winters the adult insects are active throughout the season. During colder winter the species hibernates in both the egg and adult stages.

The alfalfa hopper is little affected by natural enemies and is only reduced in numbers by the variable winter temperatures. The Sonoran redwing was found to feed upon the species.

The cleaning up of places of hibernation and the eradication of weeds, rubbish, etc., is the only known system that will reduce the numbers of the pest.

LITERATURE CITED

(1) 1831. SAY, THOMAS.
Descriptions of new North American hemipterous insects, belonging to the first family of the section Homoptera of Latreille. *In* Jour. Acad. Nat. Sci. Phila., v. 6, pt. 2, p. 235–244; 299–314. Mar., 1831.

(2) 1869. STÅL, C.
Bidrag till membracidernas kännedom. *In* Öfvers. K. Svenska Vetensk. Akad. Förhandl., bd. 26, no. 3, p. 231–300. 1869.
C. festina Say, p. 246, no. 2.

(3) 1869. STÅL, C.
Hemiptera Fabriciana. v. 2, p. 24, Stockholm, 1869. *In* K. Svenska Vetensk. Akad. Handl., bd. 8, no. 1, p. 1–130. 1869.

(4) 1888. OEMLER, A.
[Extracts from correspondence regarding a new tomato enemy in Georgia, between A. Oemler and the Division of Entomology.] *In* U. S. Dept. Agr., Div. Ent., Insect Life, v. 1, no. 2, p. 50. Aug., 1888.

(5) 1889. PROVANCHER, L. A.
Petite Faune Entomologique du Canada. v. 3, Les Hémiptères, p. 237. Quebec, June, 1889.

(6) 1890. SMITH, J. B.
Catalogue of Insects Found in New Jersey. p. 441. Trenton, 1890.

(7) 1890. VAN DUZEE, E. P.
Synonymy of the Homoptera described by Say, Harris, and Fitch. *In* Psyche, v. 5, p. 387–391. Aug., Oct., 1890.
Membracis festina, p. 389.

(8) 1894. GODING, F. W.
Bibliographical and synonymical catalogue of the described Membracidæ of North America. *In* Bul. Ill. State Lab. Nat. Hist., v. 3, p. 391–482. 1894.

(9) 1899. COCKERELL, T. D. A.
Some insect pests of Salt River Valley and the remedies for them. Ariz. Agr. Exp. Sta. Bul. 32, p. 273–295. Dec., 1899.
Stictocephala festina Say, p. 282.

(10) 1908. VAN DUZEE, E. P.
Studies in North American Membracidae. *In* Bul. Buffalo Soc. Nat. Sci., v. 9, p. 29–129. 1908.
Stictocephala festina Say, p. 45–46.

(11) 1911. OSBORN, HERBERT.
Economic importance of *Stictocephala*. *In* Jour. Econ. Ent., v. 4, no. 2, p. 137–140. Apr., 1911.

PLATE XLIII

Fig. 1.—The three-cornered alfalfa hopper (*Stictocephala festina*): Adult. *a*, view from side; *b*, view from front. Greatly enlarged. Original.

Fig. 2.—The three-cornered alfalfa hopper: *a*, Nymph in first stage; *b*, egg. Greatly enlarged. Original.

Fig. 3.—The three-cornered alfalfa hopper: Nymph in second stage. Greatly enlarged. Original.

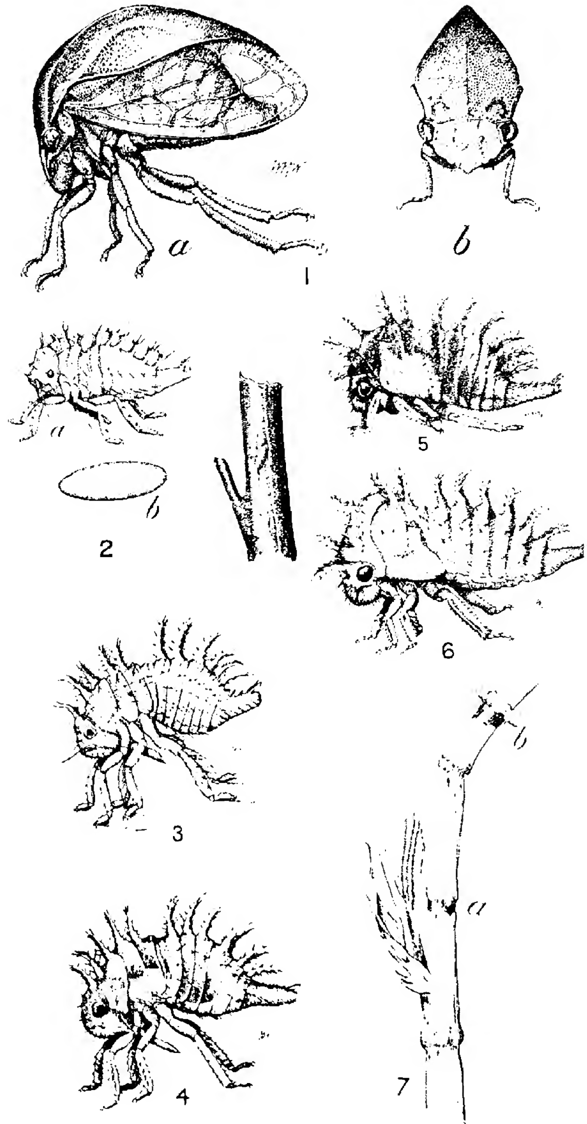
Fig. 4.—The three-cornered alfalfa hopper: Nymph in third stage. Greatly enlarged. Original.

Fig. 5.—The three-cornered alfalfa hopper: Nymph in fourth stage. Greatly enlarged. Original.

Fig. 6.—The three-cornered alfalfa hopper: Nymph in fifth stage. Greatly enlarged. Original.

Fig. 7.—An alfalfa stem showing feeding punctures of the three-cornered alfalfa hopper: *a*, Ring or girdle of punctures around the stem; *b*, gall resulting from girdling. Original.

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
25 CENTS PER COPY
SUBSCRIPTION PER YEAR, 12 NUMBERS, $2.50


a
b
1
a
b
2
5
6
3
b
a
4
7